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(54) Title: GENETIC VACCINE AGAINST HUMAN IMMUNODEFICIENCY VIRUS

(57) **Abstract:** Recombinant adenovirus and methods of administration to a host are provided for eliciting immune response of the host to human immunodeficiency virus (HIV). The recombinant adenovirus is capable of expressing multiple wild type or mutant HIV antigens such as HIV envelope proteins without the cleavage site or the cytosolic domain, structural proteins such as Gag and its proteolytical fragments in a natural, secreted or membrane-bound form, HIV enzymes such as reverse transcriptase, protease and integrase, and regulatory proteins such as Tat, Rev and Nef. Immuno-stimulators such as cytokines can also be expressed by the recombinant adenovirus to further enhance the immunogenicity of the HIV antigens.

**GENETIC VACCINE AGAINST HUMAN IMMUNODEFICIENCY VIRUS****BACKGROUND OF THE INVENTION**

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Field of the Invention

10 This invention relates to vaccines for stimulating immune responses in human and other hosts, and, in particular, relates to recombinant viruses that express heterologous antigens of human immunodeficiency virus (HIV) in a host and elicit immune response to HIV infection.

Background of the Invention

15 Current techniques for developing vaccines are largely based on the concept of using denatured virus or purified viral proteins made from bacteria. These types of vaccines may be effective for only a limited number of infectious agents, and the protection rates are limited.

20 For viruses that contain membrane (envelope) glycoproteins (GPs), including the Ebola virus and the HIV virus, use of denatured virus or purified viral proteins often does not work satisfactorily. There may be several reasons for this. First, the GPs of these viruses are sensitive to the denaturing procedures so that the epitopes of the proteins are altered by the denaturing process. Second, the sugar moieties of the GPs are important antigenic  
25 determinants for neutralizing antibodies. In comparison, proteins made in bacteria are not properly glycosylated and can fold into somewhat different structures that can have antigenicities different from those of the natural viral proteins. Further, many vaccines that are based on attenuated or denatured virus provide a weak immune response to poorly immunogenic antigens. In  
30 addition, the vaccine preparations frequently offer only limited protection, not life-long immunity as desired.

Other vaccine approaches express antigens by plasmids directly injected into the body, the so-called naked DNA or DNA vaccine technology. These methods involve the deliberate introduction of a DNA plasmid carrying  
35 an antigen-coding gene by transfecting cells with the plasmid in vivo. The plasmid expresses the antigen that causes an immune response. The immune

response stimulated by DNA vaccine can be very inefficient, presumably due to low levels of uptake of the plasmid and low levels of antigen expression in the cells. DNA vaccines are also characterized by an extremely short antigen expression period due to vector degradation. In addition, DNA vaccines are  
5 difficult and costly to produce in large amounts.

Replication-competent, live vaccinia viruses have also been modified for expression of the genes for hepatitis B (HBV), human immunodeficiency virus (HIV), influenza and malaria antigens. In some instances, though, the immune response of recombinant vaccines is often of limited nature and  
10 magnitude. Thus, for example, while peripheral immunization with vaccinia influenza recombinants provides good protection against lower respiratory tract infections, it fails to induce immunity in the upper respiratory tract. On the other hand, peripheral immunization with recombinant vaccines may prove ineffective when local rather than systemic immunity is required, as in, for  
15 example, the gastro-intestinal tract.

Vaccination with recombinant vaccinia virus expressing Ebola virus GP has been attempted to confer partial protection in guinea pigs. Gilligan, K.J., et al., *Vaccines*, 97:87-92 (1997). Vaccination with DNA constructs expressing either GP or nucleocapsid protein (NP) protects mice from lethal challenge with  
20 Ebola virus. Vanderzanden, L., et al., *Virology*, 246(1):134-44 (1998). However, each of these approaches has its own set of limitations that make them less than ideal choices for Ebola virus vaccines in humans. For example, vaccinia virus rapidly kills vector-infected cells. Consequently, the vaccine antigen is expressed for only a short time. However, the major limitation for  
25 this type of approaches is that the replication of vaccinia virus causes the immune system to react mainly to the vaccinia proteins, only small portion of the immune responses is targeted to the antigen of the pathogenic virus. This phenomenon has been termed "antigen dilution".

Previous attempts to remedy these deficiencies, including expression of vaccine antigens through viruses having stronger promoters, such as poxvirus,  
30 have not met with significant success.

As yet, no vaccine has been effective in conferring protection against HIV infection. Attempts to develop vaccines have thus far failed. Certain antibodies reactive with HIV, notably anti-GP160/120 are present at high levels  
35 throughout both the asymptomatic and symptomatic phases of the HIV infection, suggesting that rather than playing a protective role, such antibodies

may in fact promote the attachment and penetration of the virus into the host cell. More significantly, current vaccines do not induce efficient cellular responses against the infected cells, the source of newly released virions.



## SUMMARY OF THE INVENTION

Genetic viral vaccines are provided. These vaccines are designed to mimic natural infection of pathogenic viruses without causing diseases that are naturally associated with the pathogenic viruses in a host to be immunized, such as human, domestic animals and other mammals.

The vaccines are recombinant benign viruses that are replication deficient or incompetent. The benign viruses may be designed to express antigens from a wide variety of pathogens such as viruses, bacteria and parasites, and thus may be used to treat this wide variety of viruses, bacteria, and parasites that natively express these antigens. Infection of the benign virus causes host cells to express the antigens of the pathogenic virus and presents the antigen in its natural conformation and pathway as if the cell were infected by the pathogenic virus, and induces a strong and long-lasting immune response in the host.

In one embodiment, a recombinant benign virus is provided for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: an antigen sequence heterologous to the benign virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the pathogenic virus and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the benign virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. The recombinant virus is replication-incompetent and does not cause disease that is associated with the pathogenic virus in the host

In a variation of the this embodiment, the recombinant benign virus may be a replication-incompetent virus such as adenovirus, adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus or vaccinia virus. Preferably, the benign virus does not have the pathologic regions of the native progenitor of the benign virus but retains its infectivity.

In a preferred embodiment, the benign virus is a replication-incompetent adenovirus, more preferably adenovirus type 5. The heterologous antigen sequence may be positioned in the E1, E3 or E4 region of the adenovirus. The immuno-stimulator sequence may be positioned in the E4, E3 or E1 region of the adenovirus.

In a variation of the preferred embodiment, the heterologous antigen sequence and the immuno-stimulator sequence are positioned in the E1, E3 or E4 region of the adenovirus, where the heterologous antigen sequence and the immuno-stimulator sequence are expressed from a promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

Expression of the viral antigen or the immuno-stimulator may be controlled by a promoter homologous to the native progenitor of the recombinant virus. Alternatively, expression of the viral antigen may be controlled by a promoter heterologous to the native progenitor of the recombinant virus. For example, the promoter heterologous to the native progenitor of the recombinant virus may be a eukaryotic promoter such as insulin promoter, human cytomegalovirus (CMV) promoter and its early promoter, simian virus SV40 promoter, Rous sarcoma virus LTR promoter/enhancer, the chicken cytoplasmic  $\beta$ -actin promoter, and inducible promoters such as the tetracycline-inducible promoter.

The pathogenic virus may be any pathogenic virus that causes pathogenic effects or disease in human or other animals. Thus, the recombinant benign virus can be used as a vaccine for protecting the host from infection of the pathogenic virus.

In a variation, the pathogenic virus may be various strains of human immunodeficiency virus (HIV), such as HIV-1 and HIV-2. The viral antigen may be an HIV glycoprotein (or surface antigen) such as HIV GP120 and GP41, or a capsid protein (or structural protein) such as HIV P24 protein.

In another variation, the pathogenic virus may be Ebola virus. The viral antigen may be an Ebola glycoprotein or surface antigen such as Ebola GP1 or GP2 protein.

In yet another variation, the pathogenic virus may be hepatitis virus such as hepatitis A, B, C, D or E virus. For example, the viral antigen may be a surface antigen or core protein of hepatitis B virus such as the small hepatitis B surface antigen (SHBsAg) (also referred to as the Australia antigen), the middle hepatitis B surface antigen (MHBsAg) and the large hepatitis B surface antigen (LHBsAg). The viral antigen may be a surface antigen or core protein of hepatitis C virus such as NS3, NS4 and NS5 antigens.

In yet another variation, the pathogenic virus may be a respiratory syncytial virus (RSV). For example, the RSV viral antigen may be the

glycoprotein (G-protein) or the fusion protein (F-protein) of RSV, for which the sequences are available from GenBank.

In yet another variation, the pathogenic virus may be a herpes simplex virus (HSV) such as HSV-1 and HSV-2. For example, the HSV viral antigen  
5 may be the glycoprotein D from HSV-2.

In yet another variation, the viral antigen may be a tumor antigen, such as Her 2 of breast cancer cells and CD20 on lymphoma cells, a viral oncogene such as E6 and E7 of human papilloma virus, or a cellular oncogene such as mutated ras.

10 It is noted that, other virus-associated proteins or antigens are readily available to those of skill in the art. Selection of the pathogenic virus and the viral antigen associated with the pathogenic virus is not a limiting factor in this invention.

The recombinant virus also expresses an immuno-stimulator to mimic  
15 cytokine-releasing response of a host cell upon viral infection and further augments the immune response to the viral antigen co-expressed from the recombinant virus. The immuno-stimulator may preferably be a cytokine. Examples of cytokine include, but are not limited to, interleukin-2, interleukin-8, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony  
20 stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF).

The viral antigen may be a full-length antigenic viral protein or a portion of the antigenic viral protein that contains the predominant antigen, neutralizing antigen, or epitope of the pathogenic virus. Alternatively, the viral antigen  
25 contains the constant region of glycoproteins of at least two strains of the pathogenic virus.

In a variation, the viral antigen may be a modified antigen that is mutated from a glycoprotein of the pathogenic virus such that the viral antigen is rendered non-functional as a viral component but retains its antigenicity.  
30 Such modification of the viral antigen includes deletions in the proteolytic cleavage site of the glycoprotein, and duplications and rearrangement of immunosuppressive peptide regions of the glycoprotein.

In another embodiment, a recombinant adenovirus is provided for eliciting an immune response in a host infected by the virus. The recombinant  
35 virus comprises: an antigen sequence heterologous to adenovirus and encoding a viral antigen from a pathogenic virus, expression of the viral

antigen eliciting an immune response directed against the viral antigen upon infection of the host by the recombinant adenovirus.

In a preferred variation of the embodiment, the recombinant virus is a replication-incompetent adenovirus. In particular, the pathogenic virus is HIV, including various types (e.g., HIV-1 and HIV-2), strains (e.g, strain BH10 and pNL4-3 of HIV-1), isolates, clades within a group of isolates (e.g., clade A, B, C, D, E, F, and G of group M of HIV-1 isolates) of HIV. The viral antigen may be a 1) HIV glycoprotein (or surface antigen) such as HIV envelope protein Env, either full length wild type (gp160), truncated (e.g, gp120 and gp41), or modified with insertions, deletions or substitutions; 2) HIV structural protein Gag, either full length wild type, modified, or protease-processed products or fragments in various forms (e.g., natural, secreted, or membrane bound forms of HIV capsid proteins such as HIV p24 and p17; and 3) HIV regulatory proteins such as Tat, Vif, Nef, and Rev.

According to this variation, the HIV antigen is an HIV envelop protein encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 14, 16, 20, 21, 22, 23, and 24. The polynucleotide may further encode HIV regulatory proteins such as Tat, Vif, Nef, and Rev.

Also according to the variation, the HIV antigen is a modified HIV envelope protein that includes multiclade variable loops. Preferably, the multiclade variable loops are V3 loops from various clades such as clade A, B, C, D, E, F, and G of group M of HIV-1 isolates. The modified HIV envelope protein that includes multiclade variable loops may include two or more V3 loops from different HIV clades, preferably V3 loops encoded by polynucleotides selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29, 30, and 31. More preferably, the modified HIV envelope protein that includes multiclade variable loops is encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 32, 52, and 54.

Also according to the variation, the HIV antigen is an HIV structural protein. The HIV structural protein may be a full length wild type Gag encoded by SEQ ID NO: 17, or a proteolytic fragment of Gag such as p17/24, p17 and p24. The fragment p17/24 may be in natural form and encoded by SEQ ID NO: 34, in secreted form and encoded by SEQ ID NO: 34, or in membrane bound form and encoded by SEQ ID NO: 36. The fragment p17 may be in natural form and encoded by SEQ ID NO: 40, in secreted form and encoded by SEQ ID NO: 41, or in membrane bound form and encoded by SEQ ID NO: 42.

Similarly, p24 may be in natural form and encoded by SEQ ID NO: 46, in secreted form and encoded by SEQ ID NO: 47, or in membrane bound form and encoded by SEQ ID NO: 48.

5           The recombinant virus may further comprise a polynucleotide encoding an HIV protease PI such as SEQ ID NO: 56, expression of which facilitates proteolytic processing of Gag expressed from the same recombinant virus or from another vector. PI may be expressed as a fusion protein with Gag, or separately from a different promoter or from the same promoter for Gag via an IRES or splicing donor/acceptor mechanism.

10           The recombinant virus may further comprise a polynucleotide encoding a fusion protein of HIV protease PI and HIV reverse transcriptase RT, PI-RT. PI may be expressed as a fusion protein with Gag, or separately from a different promoter or from the same promoter for Gag via an IRES or splicing donor/acceptor mechanism.

15           The recombinant virus may further comprise a polynucleotide which is an HIV *Pol* gene and encodes the HIV enzyme proteins: HIV protease PI, reverse transcriptase RT, and integrase IN. Pol may be expressed as a fusion protein with Gag, or separately from a different promoter or from the same promoter for Gag via an IRES or splicing donor/acceptor mechanism.

20           Optionally, the recombinant virus may further comprise an immuno-stimulator sequence heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen.

25           The present invention also provides viral vaccines that present multiple antigens to the host to further mimic natural infection of a native pathogenic virus and induce strong and long-lasting immune response to various strains or types of the pathogenic virus in the host.

30           In one embodiment, a recombinant virus is provided as a viral vaccine for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: a plurality of antigen sequences heterologous to the recombinant virus, each encoding a viral antigen from a same pathogenic virus, different strains of a pathogenic virus, or different kinds of pathogenic viruses, expression of the plurality of the antigen sequences eliciting an immune response directed against the viral antigen and cells expressing the  
35           viral antigen in the host upon infection of the host by the recombinant virus.

The recombinant virus may preferably be replication-incompetent and not cause malignancy in the host naturally associated with pathogenic virus.

According to the embodiment, the recombinant virus may be any virus, preferably replication-incompetent adenovirus, adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus or vaccinia virus. The benign virus may also preferably have the pathologic regions of the native progenitor of the benign virus deleted but retain its infectivity.

Also according to the embodiment, the plurality of the antigen sequences may be multiple copies of the same antigen sequence or multiple antigen sequences that differ from each another.

In a variation of the embodiment, at least two of the plurality of the antigen sequences are expressed from a promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

Optionally, at least two of the plurality of the antigen sequences are expressed from a promoter to produce a fusion protein.

Also according to the embodiment, the viral genome further comprises at least one promoter heterologous to the native progenitor of the recombinant virus that controls the expression of at least two of the plurality of the antigen sequences. Examples of the promoter heterologous to the native progenitor of the recombinant virus include, but are not limited to, insulin promoter, CMV promoter and its early promoter, SV40 promoter, retrovirus LTR promoter/enhancer, the chicken cytoplasmic  $\beta$ -actin promoter, and inducible promoters such as tetracycline-inducible promoter.

Also according to the embodiment, the plurality of antigen sequences may be a combination of antigens from at least two strains of the pathogenic virus.

Optionally, the plurality of antigen sequences may be a combination of antigens from at least two different pathogenic viruses. For example, the plurality of antigen sequences may be a combination of antigens from HIV-1, HIV-2, herpes simplex virus type 1, herpes simplex virus type 2, Ebola virus, Marburg virus, Arbovirus (a group of viruses carried by mosquitoes that cause encephalitis, yellow fever, and dengue), and hepatitis A, B, C, D, and E viruses.

In a variation of the embodiment, the recombinant virus may further comprise one or more immuno-stimulator sequences that are heterologous to the benign virus and encodes an immuno-stimulator whose expression in the

host enhances the immunogenicity of the viral antigen. For example, the immuno-stimulator may be a cytokine. Examples of the cytokine include, but are not limited to, interleukin-2, interleukin-4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, G-CSF, and GM-CSF.

5           According to the variation, the one or more immuno-stimulator sequences may be multiple copies of the same immuno-stimulator sequence or multiple immuno-stimulator sequences that differ from each other.

          Optionally, at least two of the immuno-stimulator sequences may be expressed from a promoter multicistronically via an internal ribosomal entry site  
10           or via a splicing donor-acceptor mechanism. Alternatively, at least two of the immuno-stimulator sequences may be expressed from a promoter to form a fusion protein.

          The present invention also provides genetic vaccines that elicit strong and long-lasting immune response to pathogenic bacteria. In one  
15           embodiment, a recombinant virus is provided as a genetic bacteria vaccine for eliciting an immune response in a host infected by the recombinant virus. The recombinant virus comprises: a plurality of antigen sequences heterologous to the recombinant virus, each encoding a bacterial antigen from a pathogenic bacteria, expression of the plurality of the bacterial antigen sequences eliciting  
20           an immune response directed against the bacterial antigen and cells expressing the bacterial antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause malignancy naturally associated with the pathogenic bacteria in the host.

25           The pathogenic bacteria may be any pathogenic bacteria that causes pathogenic effects or diseases in a host, such as *bacillus tuberculoses*, *bacillus anthracis* (causing vegetative anthrax), and *spirochete Borrelia burgdorferi* that causes the Lyme disease in animals. The plurality of antigen sequences may encode lethal factors, protective antigen, edema factors of the  
30           pathogenic bacteria, or combinations thereof.

          The present invention also provides vaccines against parasites that elicit strong and long-lasting immune response to pathogenic parasites. In one  
embodiment, a recombinant virus is provided as a parasite vaccine for eliciting an immune response in a host infected by the recombinant virus. The  
35           recombinant virus comprises: a plurality of antigen sequences heterologous to the benign virus, each encoding a parasitic antigen from a pathogenic parasite,

expression of the plurality of the parasitic antigen sequences eliciting an immune response directed against the parasitic antigen and cells expressing the parasitic antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause a malignancy naturally associated with the pathogenic parasite in the host.

The pathogenic parasite may be any pathogenic parasites that cause pathogenic effects or diseases in a host, such as malaria and protozoa such as *Cryptosporidium*, *Eimeria*, *Histomonas*, *Leucocytozoon*, *Plasmodium*, *Toxoplasma*, *Trichomonas*, *Leishmania*, *Trypanosoma*, *Giardia*, *Babesia*, and *Theileria*. The plurality of antigen sequences may encode coat proteins, attachment proteins of the pathogenic parasites, or combinations thereof.

The present invention also provides pharmaceutical compositions that include the viral vaccines of the present invention. The pharmaceutical composition may include any of the recombinant viruses described above and a pharmaceutically acceptable carrier or diluent.

The pharmaceutical composition may also include an adjuvant for augmenting the immune response to the viral antigen expressed from the recombinant virus. Examples of the adjuvant include, but are not limited to, bacillus Calmette-Guerin, endotoxin lipopolysaccharide, keyhole limpet hemocyanin, interleukin-2, GM-CSF, and cytoxan.

The present invention also relates to kits. These kits may include any one or more vaccines according to the present invention in combination with a composition for delivering the vaccine to a host and/or a device, such as a syringe, for delivering the vaccine to a host.

The present invention also provides methods for enhancing the immunity of a host with the recombinant viruses described above.

In one embodiment, the method comprises: administering to the host a recombinant virus in an amount effective to induce an immune response. The recombinant virus comprises: an antigen sequence heterologous to the recombinant virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the benign virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral



antigen. The recombinant virus may preferably be replication-incompetent and not cause malignancy naturally associated with the pathogenic virus in the host.

5 The recombinant virus may be administered to the host via any pharmaceutically acceptable route of administration. The recombinant virus may be administered to the host via a route of intramuscular, intratracheal, subcutaneous, intranasal, intradermal, intramucosally, rectal, oral and parental administration.

10 In another embodiment, a method is provided for enhancing the immunity of a host to a pathogenic virus with multiple antigens. The method comprises: administering to the host a recombinant virus in an amount effective to induce an immune response. The recombinant virus comprises: a plurality of antigen sequences heterologous to the benign virus, each encoding a viral antigen from a pathogenic virus, expression of the plurality of the  
15 antigen sequences eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause malignancy naturally associated with the pathogenic virus in the host.

20 Optionally, the recombinant virus may further comprise one or more immuno-stimulator sequences heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen.

25 In yet another embodiment, a method is provided for enhancing the immunity of a host to a pathogenic virus by using multiple recombinant viral vaccines (or viruses). Multiple recombinant viruses may carry different antigens in each recombinant virus. The multiple recombinant viruses may be administered simultaneously or step-wise to the host.

30 The method comprises: administering to a host a first and second recombinant viruses in an amount effective to induce an immune response. The first recombinant virus comprises: an antigen sequence heterologous to the first recombinant virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon  
35 infection of the host by the recombinant virus. The second recombinant virus comprises: an immuno-stimulator sequence heterologous to the recombinant

virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. The first and second recombinant viruses may preferably be replication-incompetent and not cause a malignancy naturally associated with the pathogenic virus in the host.

5           According to the embodiment, the first and second recombinant virus may be any benign virus, such as replication-incompetent adenovirus, adeno-associated virus (AAV), SV40 virus, retrovirus, herpes simplex virus, Alpha virus, Venezuelan Equine Encephalitis (VEE) virus and vaccinia virus.

10           Optionally, both the first and second recombinant viruses may be replication-incompetent adenovirus. Also optionally, one of the first and second recombinant viruses may be recombinant adenovirus and the other may be recombinant AAV, SV40 virus, retrovirus, herpes simplex virus, Alpha virus, Venezuelan Equine Encephalitis (VEE) virus or vaccinia virus.

15           In yet another embodiment, a method is provided for enhancing the immunity of a host to a pathogen. The method comprises: administering to the host a recombinant virus and one or more immuno-stimulators. The recombinant virus may be any of the recombinant viruses described above. In particular, the recombinant virus comprises one or more antigen sequences heterologous to the recombinant virus that encode one or more antigens from  
20           the pathogen. Expression of the antigen elicits an immune response directed against the antigen and cells expressing the antigen in the host upon infection of the host by the recombinant virus. The recombinant virus is preferably replication-incompetent and does not cause a malignancy naturally associated with the pathogen in the host. The pathogen may be a pathogenic virus such  
25           as HIV, hepatitis virus and Ebola virus, a pathogenic bacteria or parasite.

          According to this embodiment, the immuno-stimulator may be any molecule that enhances the immunogenicity of the antigen expressed by the cell infected by the recombinant virus. Preferably, the immuno-stimulator is a cytokine, including, but not limited to interleukin-2, interleukin-8, interleukin-12,  
30            $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, and combinations thereof. The cytokine may be administered into the host in a form of purified protein alone or formulated with one or more pharmaceutically acceptable excipients. Alternatively, the cytokine may be administered in a form of expression vector  
35           that expresses the coding sequence of the cytokine upon transfecting or transducing the cells of the host.

According to any of the above embodiments of the methods, the method may further comprise: administering to the host the recombinant virus again to boost the immune response. Such a booster inoculation with the recombinant virus is preferably conducted several weeks to several months  
5 after the primary inoculation. To insure sustained high levels of protection against infection or an efficacious treatment of the disease(s) caused by infection of the pathogen, it may be helpful to re-administer the booster immunization to the host at regular intervals, for example, once every several years. The recombinant virus administered in the booster immunization may  
10 be the same as or different from the recombinant virus administered in the primary immunization.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a plasmid vector that encodes the same or different antigen(s) as that (or those) encoded by the  
15 recombinant virus. The plasmid vector is preferably a eukaryotic plasmid expression vector that expresses the antigen(s) upon transfection of the cells in the host.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a second recombinant  
20 virus to boost the immune response and/or to minimize neutralizing effects of the host's immune system on the recombinant viruses.

Optionally, the second recombinant virus comprises a second antigen sequence from a second pathgen that is different from the first antigen sequence comprised in the first recombinant virus administered in the primary  
25 immunization. Preferably, the second antigen sequence encodes the same type of antigen as that encoded by the first antigen sequence but from a different strain, serotype, or subtype/clade of the same pathogen.

Alternatively, the second antigen may be a different type of antigen compared to the first antigen, for example, the first antigen being a surface protein and  
30 second antigen being a core protein of the same or different pathogen.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a viral vector prior to, concurrently, or post the administration of any of the above embodiment of the recombinant virus to minimize neutralizing effects of the host's immune system  
35 on the recombinant virus. Preferably, the viral vector is administered post the administration of the recombinant virus.

The viral vector may be the native progeny of the recombinant virus. For example, the viral vector may be the wildtype adenovirus type 5 (Ad5) whereas the recombinant virus is a genetically modified Ad5.

5        Optionally, the viral vector may be the wildtype of or a genetically modified virus that is a different serotype of the recombinant virus. For example, the recombinant virus may be a genetically modified Ad5 whereas the viral vector is the wildtype of or a genetically modified adenoviral vector serotype other than Ad5, for example, serotype 1-4 or 6-51. It is noted that other serotypes discovered and/or classified later also fall within the scope of  
10      the invention.

Also optionally, the viral vector may be a different virus from the recombinant virus. For example, the recombinant virus may be a genetically modified Ad5 whereas the viral vector is a genetically modified AAV, SV40 virus, retrovirus, herpes simplex virus, Alpha virus, Venezuelan Equine  
15      Encephalitis (VEE) virus or vaccinia virus. The viral vector may or may not comprise a heterologous antigen sequence. Preferably, the viral vector may comprise another antigen sequence which is the same or different from the antigen sequence carried by the recombinant virus.

Also optionally, the viral vector may be a chimeric vector modified  
20      based on the native progenitor of the recombinant virus. For example, if the native progenitor of the recombinant virus is adenovirus type 5, the viral vector may be a chimeric adenovirus type 5 with certain regions of the backbone changed from type 5 to the corresponding regions from other adenovirus serotypes. For example, the fiber knob, shaft, and/or penton base in the  
25      backbone of adenovirus type 5 can be replaced by the corresponding region(s) of the backbone from adenovirus serotype 1-4, and 6-51.

Also optionally, the viral vector also comprises one or more heterologous antigen sequence and/or immuno-stimulator sequence which are the same or different from those in the recombinant virus.

30        The methods described above may be used for prevention or treatment of diseases. In the method of treatment, the administration of the recombinant viruses of the present invention may be for either "prophylactic" or "therapeutic" purpose. When provided prophylactically, the recombinant virus is provided in advance of any symptom. The prophylactic administration of the recombinant  
35      virus serves to prevent or ameliorate any subsequent infection or disease. When provided therapeutically, the recombinant virus is provided at (or after)

the onset of a symptom of infection or disease. Thus, the present invention may be provided either prior to the anticipated exposure to a disease-causing agent or after the initiation and/or progression of the infection or disease.

5 It is noted that the innovative approaches of the present invention may also be employed in construction of cancer vaccines. For example, sequences encoding tumor-specific antigens may substitute the antigen sequence encoding viral antigen in any of the above embodiments of the recombinant virus and methods of using the same. Expression of tumor-specific antigens in the host should elicit specific immune response for prevention in patients with  
10 an increased risk of cancer development (i.e., preventive immunization) or to enhance the treatment of cancer with other therapeutics, prevention of disease recurrence after primary surgery (anti-metastatic vaccination), or as a tool to expand the number of CTL in vivo, thus improving their effectiveness in eradication of diffuse tumors (treatment of established disease). In addition,  
15 the methods of the present invention may elicit an immune response in a patient that is enhanced ex vivo prior to being transferred back to the tumor bearer (i.e., the adoptive immunotherapy).

Also according to any of the above embodiments of the methods, the method may further comprise: harvesting serum from the host after the  
20 administration of the recombinant virus. The harvested serum should contain antibodies against the antigen(s) encoded by the recombinant virus. Optionally, the method may further comprise: isolating antibody or antibodies against the pathogen from the host after the administration of the recombinant virus. The harvested serum or isolated antibody can be stored for certain  
25 periods of time for further uses. For example, a healthy human volunteer can serve as the host and the serum or antibody collected from him/her may be administered back to him/herself or a different person later to in anticipation or in the event of infection of the pathogen as prophylactic or therapeutic agent by eliciting passive immunity against the pathogen. Optionally, the host may be a  
30 non-human animal and the serum harvested or antibody isolated from the animal immunized by the recombinant virus may be used as a prophylactic or therapeutic agent to treat a human or non-human animal in anticipation or in the event of infection of the pathogen such as in the outbreak of biological warfare.

35 It should be noted that modifications and changes can be made in the DNA sequence of any of the above-described antigens and immuno-

stimulators included in the recombinant virus and still maintain functional equivalence of the mutant. For example, wildtype codons for the above-described antigens can be replaced with codons that are preferred by the host to be immunized, e.g., a human. The resulting mutants fall within the scope of the present invention.

## BRIEF DESCRIPTION OF THE FIGURES

**Figures 1A-1C** illustrate an example of how to construct a genetic vaccine of the present invention.

**Figure 1A** illustrates an example of a shuttle vector pLAd.Antigen carrying multiple antigen genes such as Antigen 1 and Antigen 2 which can be expressed from a CMV<sub>ie</sub> promoter bicistronically via a splicing donor-acceptor mechanism at the SD and SA sites.

**Figure 1B** illustrates an example of a shuttle vector pRAd.Cytokines carrying multiple cytokine genes such as IL-2, INF, and IL-8 genes which can be expressed from a CMV<sub>ie</sub> promoter bicistronically via an internal ribosomal entry site IRES and a splicing donor-acceptor mechanism at the SD and SA sites.

**Figure 1C** illustrates an example of constructing a genetic vaccine by ligating with an adenoviral backbone with a fragment that is derived from the shuttle vector pLAd.Antigen and contains multiple antigen genes and a fragment that is derived from the shuttle vector pRAd.Cytokines and contains multiple cytokine genes.

**Figure 2** illustrates the wild-type GP gene, which encodes the two forms of glycoproteins (sGP and GP), contains a RNA editing signal that results in un-edited and edited mRNAs. The sGP is synthesized from an un-edited mRNA and the GP is synthesized from an edited mRNA (having an insertion in one of the seven uridines). **Figure 2** also depicts the modifications made to the RNA to prevent the synthesis of sGP. The RNA editing site is modified from UUU UUU U to UUC UUC UU. This modification removes the editing signal and results in the mRNA coding only for the GP.

**Figure 3** illustrates the modification of the immunosuppressive peptide (IS) located in GP2. **Figure 3A** shows the wild type GP. **Figure 3B** shows GP with the 10 amino acid deletion of the IS peptide. **Figure 3C** shows the IS

peptide, which is split, reversed and duplicated. Abbreviations: FP, Fusion peptide; IS, Immunosuppressive peptide; TM, Transmembrane domain.

**Figures 4A and 4B** illustrate a procedure used to create a recombinant adenoviral vector as a genetic vaccine against Ebola virus.

5        **Figures 4A** illustrates a shuttle vector pLAd/EBO-GP carrying the GP gene of Ebola virus an antigen, and a shuttle vector pRAdIL2,4 carrying the IL-2 and IL-4 gene.

10        **Figure 4B** illustrates the construction of a recombinant adenoviral vector by ligating an adenoviral backbone with a fragment that is derived from the shuttle vector pLAd/EBO-GP and contains the GP gene and a fragment that is derived from the shuttle vector pRAdIL2,4 and contains IL-2 and IL-4 genes.

15        **Figure 5** illustrates a complex adenoviral vector as an example of the genetic vaccine of the present invention. The Ebola viral GP gene is expressed by a CMVie promoter in the E1 region. The GP gene is followed by INF- $\gamma$  and GM-CSF which are expressed by two IRES sequences. This configuration allows for the expression of three proteins from a single mRNA. Expression of IL-2 and IL-4 is controlled by a second CMVie promoter as a bi-cistronic cassette, and followed by a second bi-cistronic cassette that  
20        expressed the two subunits of IL12 in the E4 region by a SV40 early promoter.

**Figure 6** shows relative titers of antibody against HIV antigens in a group of mice.

**Figure 7** shows relative titers of antibody against HIV antigens in another group of mice.

25        **Figure 8A-C** show INF- $\gamma$  secretion from activated splenocytes harvested from mice inoculated with adenoviral vectors in response to target cell stimulation.

30        **Figure 9** shows granzyme A secretion from activated splenocytes harvested from mice inoculated with adenoviral vectors in response to target cell stimulation.

**Figure 10A** shows relative titers of antibody against HBV surface antigen in a group of mice.

**Figure 10B** shows relative titers of antibody against HBV surface antigen in another group of mice.

**Figure 11A** shows relative titers of antibody against HBV core antigen in a group of mice.

**Figure 11B** shows relative titers of antibody against HBV core antigen in another group of mice.

5        **Figure 12A** shows relative titers of antibody against HIV Gag in mice in week 10 post-immunization with Ad-3C/E<sup>m</sup>ΔCΔT<sup>300</sup>-G.

**Figure 12B** shows relative titers of antibody against HIV Gag in mice in week 14 post-immunization/week 3 post-boost with Ad-3C/E<sup>m</sup>ΔCΔT<sup>300</sup>-G.

10       **Figure 13A** shows relative titers of antibody against HIV Gag in mice in week 10 post-immunization with Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>-G.

**Figure 13B** shows relative titers of antibody against HIV Gag in mice in week 14 post-immunization/week 3 post-boost with Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>-G.

15       **Figure 14A** shows results of the granzyme A assays for serie 1 mice at week 4, 6, 8 post-immunization and week 12/1, 13/2, 14/3 (prime/boost) post-secondary inoculation with Ad.3C.env.gag.

**Figure 14B** shows the results of the granzyme A assays for serie 2 mice at week 2, 4, 6, 8 post-immunization with Ad.3C.env.gag.

**Figure 15A** shows the ELISPOT results for the four mice in serie 1 at week 13/2 post-prime/boost with Ad.3C.env.gag.

20       **Figure 15B** shows the ELISPOT results for the four mice in serie 1 at week 13/2 post-prime/boost with Ad.3C.env.rev.gag.

**Figure 16A** illustrates a shuttle vector pLAd-E.T.R.

**Figure 16B** illustrates a shuttle vector pRAd-ORF6-IL2.

**Figure 17A** illustrates a shuttle vector pRAd-ORF6-cmv- E<sup>m</sup>ΔCΔT<sup>300</sup>-G.

25       **Figure 17B** illustrates a shuttle vector pLAd-3C.

**Figure 18** illustrates a shuttle vector pRAd-E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R-G

**Figure 19A** illustrates a shuttle vector pLAd- E<sup>m</sup>ΔV<sub>1,2</sub> ΔC ΔT.T.R-IL2.

**Figure 19B** illustrates a shuttle vector pRAd-ORF6-G.IL2.

**Figure 20** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔC.T.R.N.

30       **Figure 21** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔC.N.

**Figure 22** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔC ΔT<sup>300</sup>.T.

**Figure 23A** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔC.

**Figure 23B** illustrates a shuttle vector pRAd-ORF6-E<sup>m</sup>ΔC.



**Figure 24** illustrates a process for constructing a multi-clade insert by PCR.

**Figure 25** illustrates a shuttle vector pLAd-E<sup>m</sup>.V3.

**Figure 26** illustrates a shuttle vector pLAd-E<sup>m</sup>.2xV3.

5 **Figure 27A** illustrates a shuttle vector pRAd-ORF6-p17/p24.

**Figure 27B** illustrates a shuttle vector pRAd-ORF6-p17/p24sec.

**Figure 27C** illustrates a shuttle vector pRAd-ORF6-p17/p24MB.

**Figure 28A** illustrates a shuttle vector pRAd-ORF6-p17.

**Figure 28B** illustrates a shuttle vector pRAd-ORF6-p17sec.

10 **Figure 28C** illustrates a shuttle vector pRAd-ORF6-p17MB.

**Figure 29A** illustrates a shuttle vector pRAd-ORF6-p24.

**Figure 29B** illustrates a shuttle vector pRAd-ORF6-p24sec.

**Figure 29C** illustrates a shuttle vector pRAd-ORF6-p24MB.

15 **Figure 30A-B** illustrate a process of construction of Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17/p24MB.

**Figure 31A-B** illustrate a process of construction of Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17MB.

**Figure 32A-B** illustrate a process of construction of Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p24MB.

20 **Figure 33** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔCAT<sup>300</sup>.2xV3<sup>m</sup>.T.

**Figure 34** illustrates a shuttle vector pLAd-E<sup>m</sup>ΔCAT<sup>99</sup>.2xV3<sup>m</sup>.T.R.

**Figure 35** illustrates a shuttle vector pRAd-ORF6-G.PI.

**Figure 36** illustrates a shuttle vector pRAd-ORF6-G.PI.

**Figure 37** illustrates a cloning vector SD/SA1.2.3

25 **Figure 38** shows DNA sequence encoding Env/Tat/Rev from HIV-1 strain BH10.

**Figure 39** shows DNA sequence encoding a mutated IL-2 (IL-2ΔX).

**Figure 40** shows DNA sequence encoding a modified Env (E<sup>m</sup>ΔCAT (BH10)).

30 **Figure 41A** shows DNA sequence encoding the full length HIV Gag.

**Figure 41B** shows amino acid sequence of the full length HIV Gag.

**Figure 42** shows DNA sequence encoding Env, and full length Tat and Rev.

**Figure 43** shows DNA sequence encoding E<sup>m</sup>ΔV<sub>1,2</sub> ΔC ΔT.T.R.

**Figure 44** shows DNA sequence encoding E<sup>m</sup>ΔC.T.R.N.

**Figure 45** shows DNA sequence encoding E<sup>m</sup>ΔC.N.

**Figure 46** shows DNA sequence encoding E<sup>m</sup>ΔCΔT<sup>300</sup>.T.

**Figure 47** shows DNA sequence encoding E<sup>m</sup>/ E<sup>m</sup>.

5       **Figure 48** shows DNA sequences of V3 loops of clade B, A, C, D, E, F, and G.

**Figure 49A** shows DNA sequence encoding a modified Env including multi-clade V3 loops.

10       **Figure 49B** shows amino acid sequence encoding a modified Env including multi-clade V3 loops.

**Figure 50A** shows DNA sequence encoding p17/p24 in natural form, secreted form, and membrane bound form, respectively.

**Figure 50B** shows amino acid sequence of p17/p24 in natural form, secreted form, and membrane bound form, respectively.

15       **Figure 51A** shows DNA sequence encoding p17 in natural form, secreted form, and membrane bound form, respectively.

**Figure 51B** shows amino acid sequence of p17 in natural form, secreted form, and membrane bound form, respectively.

20       **Figure 52A** shows DNA sequence encoding p24 in natural form, secreted form, and membrane bound form, respectively.

**Figure 52B** shows amino acid sequence of p24 in natural form, secreted form, and membrane bound form, respectively.

**Figure 53A** shows DNA sequence encoding a modified Env including multi-clade V3 loops, and Tat.

25       **Figure 53B** shows amino acid sequence of a modified Env including multi-clade V3 loops, and Tat.

**Figure 54A** shows DNA sequence encoding a modified Env including multi-clade V3 loops, Tat, and Rev.

30       **Figure 54B** shows amino acid sequence of a modified Env including multi-clade V3 loops, Tat, and Rev.

**Figure 55A** shows DNA sequence encoding an HIV protease PI.

**Figure 55B** shows amino acid sequence of an HIV protease PI.

**Figure 56A** shows DNA sequence encoding HIV Gag-PI.

**Figure 56B** shows amino acid sequence of HIV Gag-PI.

**Figure 57** shows PCR primers for cloning V3 loops from multiple HIV clades.

**Figure 58** illustrates a shuttle vector pRAd-ORF6-Gag/PI-RT.

**Figure 59** illustrates a shuttle vector pRAd-ORF6-Gag-PI-RT.

5 **Figure 60** illustrates a shuttle vector pRAd-ORF6-Gag-Pol.

**Figure 61** illustrates a shuttle vector pRAd-ORF6-Gag-Pol.

**Figure 62** illustrates a right shuttle vector pR-Ad.5/35-6m.

10 **Figure 63** shows various embodiments of chimeric vectors having the individual domains of the Ad5 fiber regions substituted with the corresponding domains of Ad35 fiber region.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides genetic vaccines, pharmaceutical compositions including the vaccines and methods of immunizing a host against infection of a wide range of pathogenic viruses, bacteria and parasites. The genetic vaccines are recombinant benign viruses that are replication deficient and do not cause malignancy in the host to be immunized. Vaccination using the genetic vaccines of the present invention mimics natural viral infection in that the antigen(s) expressed by the cell infected by the genetic vaccine is presented to the host immune system in its natural conformation and by a "inside-out" mechanism, as compared with the conventional "outside-in" approach of vaccination using denatured protein or virus as a vaccine. The recombinant virus is capable of expressing multiple pathogenic antigens, mimicking natural pathogen infection. In particular, multiple pathogenic antigens such as a combination of an HIV envelop protein Env and structural protein Gag, either wildtype or mutant, can be expressed by the recombinant virus to elicit not only humoral immune response (i.e., production of antibody from B cells, helper T cells, and suppressor T cells), but also cellular response by producing cytotoxic T lymphocytes (CTL) directed specifically to these antigens. Further, the pathogenic antigen that is naturally expressed as an intracellular protein can be modified to be secretable and rendered bound to the cell surface, thus better presenting the antigen to the body's immune system. In addition, the cell infected by the genetic vaccine may also release high levels of cytokine, thereby further mimicking the natural response of the cell under stress induced by viral infection and yet not causing pathogenic effects on the cells. Mistaken by such a "signal of pathogenic viral infection", the host immune system mounts a strong immune defense against the antigen presented by the infected cell. Therefore, in a sense, the genetic vaccine of the present invention behaves like a "sheep in wolf's clothing", presenting the viral antigen to induce a strong immune response and yet not causing the detrimental effects that the pathogens would cause on the host. The recombinant viruses of the present invention can not only be used as a vaccine to prevent infection of the pathogen but also as a therapeutic agent to treat diseases associated with the infection of the pathogen.

In one embodiment, a recombinant virus is provided for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: an antigen sequence heterologous to the recombinant virus and encoding a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the recombinant virus and encoding an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. The recombinant virus is replication-incompetent and does not cause the malignancy naturally associated with the pathogenic virus in the host.

In another embodiment, a recombinant virus is provided as a viral vaccine for eliciting an immune response against multiple antigens in a host infected by the virus. The recombinant virus comprises: a plurality of antigen sequences heterologous to the benign virus, each encoding a different viral antigen from one or more pathogenic viruses, expression of the plurality of the antigen sequences eliciting an immune response directed against the viral antigens and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause the malignancy that is naturally associated with the pathogenic virus(es) in the host.

The vaccines of the present invention can be used to immunize the host against a wide variety and different strains of pathogenic viruses such as HIV-1, HIV-2, herpes simplex virus type 1, herpessimplex virus type 2, Ebola virus, Ebola virus, and hepatitis A, B, C, D, and E viruses, or pathogenic bacteria such as *bacillus tumerculoses* and *bacillus anthracis*.

The recombinant vaccine of the present invention is a recombinant virus that contains nucleic acid sequences encoding one or more viral antigens in the viral genome. When a host is immunized by the recombinant vaccine, i.e., infected by the recombinant virus, the infection of the virus in a host cell results in expression of the viral antigen which is present on the surface of the infected cell. Since expression of the viral antigen is driven by a strong promoter, expression can be maintained at a high level. Upon recognizing the large amount viral antigen on the cell surface, the host immune system mounts a strong defense against the viral antigen, thereby

achieving long-lasting immunity against the pathogenic virus from which the viral antigen is derived.

Compared with immunization with vaccines that are isolated proteins expressed by bacteria, yeast or insect cells, the viral antigen expressed from the recombinant virus of the present invention better mimics the natural viral antigen in its structure and function. Isolated protein vaccine may not adopt the native conformation of the natural viral antigen and may not be properly glycosylated in the bacteria, yeast or insect cells. When such an isolated protein vaccine is injected into the host, this antigen is presented from the outside of the host cell. This conventional "outside-in" approach often does not generate strong, long-lasting immune response, presumably due to the altered antigenicity of the vaccine and quick clearance of the protein vaccine by the immune scavenging cells.

In contrast, the genetic vaccine of the present invention, i.e., the recombinant virus, presents the viral antigen by an "inside-out" mechanism. The viral antigen is expressed after infection of the recombinant virus in the host cells. This better mimics the natural production and presentation of the viral antigen by the pathogenic virus.

By using a replication incompetent virus that is incapable of spreading beyond initially infected cells, the present invention dramatically reduces the risk of side effects that may potentially be generated by using replication-competent, live virus. For example, vaccines based on live vaccinia virus can replicate in the host cells, which can impose a high level of stress on the host cell and eventually lead to cell death.

Moreover, compared to the approach of using attenuated or inactive virus as a vaccine, the process of making the genetic vaccine of the present invention is much safer. Vaccination of a large population of people or animals demand large amounts of vaccines. For virulent viruses such as Ebola virus and HIV, large-scale production of attenuated or inactive virus from the live virus can pose a great danger to the environment and people who handle the live virus.

The recombinant virus of the present invention can be used to express multiple antigen sequences simultaneously from the same viral vector. Thus, the recombinant virus may encode multiple antigens from the same strain of pathogenic virus, from different strains of the same pathogenic viruses, or

from different antigens from different kind of viruses, bacteria or parasites. This enables the vaccines of the present invention to be utilized to immunize against a broad-spectrum of viruses and other infectious agents. Since these multiple antigen sequences are rearranged in the recombinant viral genome, the risk of potential recombination of these viral sequences to generate a pathogenic virus is virtually eliminated.

The genetic vaccine of the present invention also preferably express large amount of immuno-stimulator, such as cytokine. In a natural process of viral infection, virus-infected cells display viral antigens on their surface in the context of the MHC-I receptor, while viral particles are digested by the professional antigen-presenting cells which display antigens in association with MHC-II receptors. In response to viral infection, a full range of cytokines and interferons are produced, resulting in a strong humoral and cellular response to the viral antigens. At the same, large numbers of memory cells remain to defeat any new infection. In vaccinations using isolated protein vaccines, the protein is quickly cleared by the immune scavenging cells. During this process, only MHC-II antigen presentation occurs and the cytokine-releasing response is absent or greatly diminished. As a result, little cellular response is generated and few "memory" cells are produced.

In comparison, co-expression of viral antigen and cytokine from the recombinant virus of the present invention effectively mimics the natural response of the host cell to viral infection by presenting the antigen on the surface of the infected and producing large amount of immuno-modulating cytokines. With the high levels of cytokine expressed from the host cells infected by the genetic vaccine, the host immune system would be "tricked" to mount a strong response to vaccine, thereby resulting in a longer-lasting immunity.

Additionally, although vaccination with the genetic vaccine mimics the natural viral infection of a pathogenic virus, the vaccine itself is a benign virus that does not have the detrimental effects of the pathogenic virus. For example, infection of a pathogenic virus such as HIV, influenza virus and Ebola virus has profound immuno-suppressing effects on the host, presumably due to the immuno-suppressing functions of the glycoproteins of the virus. According to the present invention, the viral antigen sequence carried by the genetic vaccine is preferred to have its pathogenic or immuno-

suppressing regions deleted. In a sense, the genetic vaccine of the present invention behaves like a "sheep in wolf's clothing", presenting the viral antigen to induce strong immune response and yet not causing detrimental effects on the host.

5

### 1. The Genetic Vaccines of the Present Invention

The present invention is directed to vaccines that mimic the features of a native pathogenic virus, but without eliciting immuno-suppression and pathogenicity, thus causing the host to mount an effective defense, while not being in any actual danger of infection. The genetic vaccines are replication incompetent or defective viruses into which one or more DNA sequences encoding one or more viral antigens are inserted into the regions of the viral genome non-essential to its infectivity. The recombinant virus expresses the viral antigens and elicits a cell-mediated immune response *in vivo* directed against the antigens and cells expressing the antigens.

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In one embodiment, a recombinant virus is provided for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: an antigen sequence heterologous to the recombinant virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. The recombinant virus is replication-incompetent and does not cause a malignancy naturally associated with the pathogenic virus in the host.

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The recombinant virus may be constructed from any virus as long as the native progenitor is rendered replication incompetent. For example, replication-incompetent adenovirus, adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus or vaccinia virus may be used to generate the recombinant virus by inserting the viral antigen into the region non-essential to the infectivity of the recombinant virus. Therefore, it is preferred that the recombinant virus does not have the pathologic regions of the native progenitor of the benign virus but retains its infectivity to the host.

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In a preferred embodiment, the recombinant virus is a replication-incompetent adenovirus.

5 The recombinant adenovirus of the present invention can direct high levels of antigen expression that provide strong stimulation of the immune system. The antigen expressed by cells infected by adenovirus is processed and displayed in the infected cells in a way that mimics pathogen-infected cells. This phase is believed to be very important in inducing cellular immunity against infected cells, and is completely lacking when conventional  
10 vaccination approaches are used. Further, the recombinant adenovirus may infect dendritic cells which are very potent antigen-presenting cells. Further, the recombinant adenovirus may also carry genes encoding immuno-enhancing cytokines to further boost immunity. Moreover, the recombinant adenovirus may naturally infect airway and gut epithelial cells in humans, and therefore the vaccine may be delivered through nasal spray or oral ingestion.  
15 In addition, the recombinant adenovirus of the present invention should be safe because it is replication-incompetent.

The heterologous antigen sequence may be positioned in the E1, E3 or E4 region of the adenovirus. The immuno-stimulator sequence may be positioned in the E1, E3 or E4 region of the adenovirus.

20 In a variation of the preferred embodiment, the heterologous antigen sequence and the immuno-stimulator sequence are positioned in the E1, E3 or E4 region of the adenovirus, where the heterologous antigen sequence and the immuno-stimulator sequence are expressed from a promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor  
25 mechanism.

The expression of the viral antigen or the immuno-stimulator may be controlled by a promoter homologous to the native progenitor of the recombinant virus. Alternatively, the expression of the viral antigen may be controlled by a promoter heterologous to the native progenitor of the  
30 recombinant virus. For example, the promoter heterologous to the native progenitor of the recombinant virus may be a eukaryotic promoter such as insulin promoter, human cytomegalovirus (CMV) promoter and its early promoter, simian virus SV40 promoter, Rous sarcoma virus LTR promoter/enhancer, the chicken cytoplasmic  $\beta$ -actin promoter, and inducible  
35 promoters such as the tetracycline-inducible promoter.

The pathogenic virus may be any pathogenic virus that causes pathogenic effects or disease in a host such as human, domestic animals or other mammals. Thus, the recombinant virus can be used as a vaccine for protecting the host from infection of the pathogenic virus.

5           In a variation, the pathogenic virus may be various strains of human immunodeficiency virus (HIV), such as HIV-1 and HIV-2. The viral antigen may be a HIV glycoprotein (or surface antigen) such as HIV GP120 and GP41, a capsid protein (or structural protein) such as HIV P24 protein, or other HIV regulatory proteins such as Tat, Vif and Rev proteins.

10           In another variation, the pathogenic virus may be influenza virus. The viral antigen may be an influenza glycoprotein such as influenza HA1, HA2 and NA.

          In another variation, the pathogenic virus may be Ebola virus. The viral antigen may be an Ebola glycoprotein or surface antigen such as Ebola  
15   GP1 and GP2 protein.

          In yet another variation, the pathogenic virus may be hepatitis virus such as hepatitis A, B, C, D or E virus. The viral antigen may be a surface antigen or core protein of hepatitis A, B, C, D or E virus. For example, the viral antigen may be a surface antigen or core protein of hepatitis B virus such  
20   as the small hepatitis B surface antigen (SHBsAg) (also referred to as the Australia antigen), the middle hepatitis B surface antigen (MHBsAg) and the large hepatitis B surface antigen (LHBsAg). The viral antigen may also be a surface antigen or core protein of hepatitis C virus such as NS3, NS4 and NS5 antigens.

25           In yet another variation, the pathogenic virus may be a respiratory syncytial virus (RSV). For example, the RSV viral antigen may be the glycoprotein (G-protein) or the fusion protein (F-protein) of RSV, for which the sequences are available from GenBank.

          In yet another variation, the pathogenic virus may be a herpes simplex  
30   virus (HSV) such as HSV-1 and HSV-2. For example, the HSV viral antigen may be the glycoprotein D from HSV-2.

          In yet another variation, the viral antigen may be a tumor antigen or viral oncogene such as E6 and E7 of human papilloma virus, or cellular oncogenes such as mutated ras or p53.

It is noted that, other virus-associated proteins or antigens are readily available to those of skill in the art. Selection of the pathogenic virus and the viral antigen is not a limiting factor in this invention.

5 The viral antigen may be a full-length antigenic viral protein or a portion of the antigenic viral protein that contains the predominant antigen, neutralizing antigen, or epitope of the pathogenic virus. Alternatively, the viral antigen contains the conserved region of glycoproteins between at least two strains of the same pathogenic virus.

10 In a variation, the viral antigen may be a modified antigen that is mutated from a glycoprotein of the pathogenic virus such that the viral antigen is rendered non-functional as a viral component but retains its antigenicity. Such modification of the viral antigen includes deletions in the proteolytic cleavage site of the glycoprotein, and duplications and rearrangement of immunosuppressive peptide regions of the glycoprotein.

15 The recombinant virus also expresses an immuno-stimulator to mimic cytokine-releasing response of a host cell upon viral infection and further augments immune response to the viral antigen co-expressed from the recombinant virus. The immuno-stimulator may preferably be a cytokine. Examples of cytokine include, but are not limited to, interleukin-2, interleukin-20 4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF).

25 In another embodiment, a recombinant virus is provided for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: an antigen sequence heterologous to the recombinant virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus.

30 According to this embodiment, the recombinant virus is preferably be replication-incompetent adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus or vaccinia virus. The benign virus may preferably have the pathologic regions of the native progenitor of the benign virus deleted but retains its infectivity to the host.

Optionally, the recombinant virus includes an immuno-stimulator sequence heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen.

5           The present invention also provides genetic vaccines that elicit strong and long-lasting immune response to pathogenic bacteria. In one embodiment, a recombinant virus is provided as a genetic bacteria vaccine for eliciting an immune response in a host infected by the recombinant virus. The viral genome of the recombinant virus comprises: a plurality of antigen  
10 sequences heterologous to the recombinant virus, each encoding a bacterial antigen from a pathogenic bacteria, expression of the plurality of the bacterial antigen sequences eliciting an immune response directed against the bacterial antigen and cells expressing the bacterial antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be  
15 replication-incompetent and not cause a malignancy naturally associated with the pathogenic bacteria in the host.

          The pathogenic bacteria may be any pathogenic bacteria that causes pathogenic effects or diseases in a host, such as *bacillus tuberculoses*, *bacillus anthracis*, and *spirochete Borrelia burgdorferi* that causes the Lyme  
20 disease in animals. The plurality of antigen sequences may encode lethal factors, protective antigen, edema factors of the pathogenic bacteria, or combination thereof.

          The present invention also provides parasites vaccines that elicit strong and long-lasting immune response to pathogenic parasites. In one  
25 embodiment, a recombinant virus is provided as a parasite vaccine for eliciting an immune response in a host infected by the benign virus. The recombinant virus comprises: a plurality of antigen sequences heterologous to the recombinant virus, each encoding a parasitic antigen from a pathogenic parasite, expression of the plurality of the parasitic antigen sequences eliciting  
30 an immune response directed against the parasitic antigen and cells expressing the parasitic antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not a cause malignancy naturally associated with the pathogenic parasite in the host.

The pathogenic parasite may be any pathogenic parasite that causes pathogenic effects or diseases in a host, such as malaria and protozoa such as Cryptosporidium, Eimeria, Histomonas, Leucocytozoon, Plasmodium, Toxoplasma, Trichomonas, Leishmania, Trypanosoma, Giardia, Babesia, and Theileria. The plurality of antigen sequences may encode coat proteins, attachment proteins of the pathogenic parasites, or combinations thereof.

The present invention also provides viral vaccines that present multiple antigens to the host to further mimic natural infection of a native pathogenic virus and induce strong and long-lasting immune response to various strains or types of the pathogenic virus in the host.

In one embodiment, a recombinant virus is provided as a viral vaccine for eliciting an immune response in a host infected by the virus. The recombinant virus comprises: a plurality of antigen sequences heterologous to the recombinant virus, each encoding a viral antigen from a pathogenic virus, expression of the plurality of the antigen sequences eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause a malignancy naturally associated with the pathogenic virus in the host.

According to the embodiment, the recombinant virus may be any virus, preferably replication-incompetent adenovirus, adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus or vaccinia virus. The recombinant virus may also preferably have the pathologic regions of the native progenitor of the benign virus deleted but retain its infectivity to the host.

Also according to the embodiment, the plurality of the antigen sequences may be multiple copies of the same antigen sequence or multiple antigen sequences that differ from each another.

In a variation of the embodiment, at least two of the plurality of the antigen sequences are expressed from a promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

Alternatively, at least two of the plurality of the antigen sequences are expressed from a promoter to form a fusion protein.

Also according to the embodiment, the recombinant virus further comprises at least one promoter heterologous to the native progenitor of the recombinant virus that controls the expression of at least two of the plurality of

the antigen sequences. Examples of the promoter heterologous to the native progenitor of the recombinant virus include, but are not limited to, insulin promoter, CMV promoter and its early promoter, SV40 promoter, Rous sarcoma virus LTR promoter/enhancer, the chicken cytoplasmic  $\beta$ -actin promoter, and inducible promoters such as tetracycline-inducible promoter.

Also according to the embodiment, the plurality of antigen sequences may be a combination of antigens from at least two strains of the pathogenic virus.

Optionally, the plurality of antigen sequences may be a combination of antigens from at least two different pathogenic viruses. For example, the plurality of antigen sequences may be a combination of antigens from HIV-1, HIV-2, herpes simplex virus type 1, herpes simplexvirus type 2, influenza virus, Marburg virus, Ebola virus, Arbovirus (a group of viruses carried by mosquitoes that cause encephalitis, yellow fever, and dengue), and hepatitis A, B, C, D, and E viruses.

In a variation of the embodiment, the viral genome of the recombinant virus may further comprise one or more immuno-stimulator sequences that is heterologous to the recombinant virus and encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. For example, the immuno-stimulator may be a cytokine. Examples of the cytokine include, but are not limited to, interleukin-2, interleukin-4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, G-CSF, and GM-CSF.

According to the variation, the one or more immuno-stimulator sequences may be multiple copies of the same immuno-stimulator sequence or multiple immuno-stimulator sequences that differ from each other.

Optionally, at least two of the immuno-stimulator sequences may be expressed from a promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism. Alternatively, at least two of the immuno-stimulator sequences may be expressed from a promoter to form a fusion protein.

The DNA sequence encoding viral antigen(s) is inserted into any non-essential region of the replication defective virus. In the case of adenovirus, for example, the nucleic acid is preferably inserted into the E1, E3 and/or E4 region of the adenovirus and most preferably into the E4 region. Because the

E1, E3 and E4 regions are available as insertion sites, the present invention also contemplates separate insertion of more than one encoding sequence.

In the recombinant viral vector vaccines of the present invention, the selected nucleotide sequences of the viral antigens are operably linked to control elements that direct transcription or expression thereof in the subject *in vivo*. Either homologous or heterologous viral control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding hostian or viral genes. Examples include, but are not limited to a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMV<sub>ie</sub>), SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (AdMLP), a herpes simplex virus promoter, and a retrovirus LTR promoter. Preferably, any strong constitutive promoter may be operatively linked to viral antigens or cytokines. More preferably the viral promoter is CMV immediate early promoter (CMV<sub>ie</sub>).

**Figures 1A-1C** illustrate a method for constructing a recombinant adenoviral vector as a genetic vaccine of the present invention. The recombinant adenoviral vector of the present invention is constructed by using shuttle plasmids or vectors carrying multiple antigen genes and multiple cytokine genes.

**Figure 1A** illustrates a shuttle plasmid (pLAd.Antigen) containing two antigen genes, Antigen 1 and Antigen 2.. The shuttle plasmid pLAd.Antigen contains the left end of the adenoviral genome including the left long terminal repeats L-TR, and an adenoviral packaging signal ( $\psi$ ). The E1 region of the adenovirus is replaced by a multiple gene expression cassette and CMV<sub>ie</sub> promoter.

Genes encoding Antigen 1 and Antigen 2 are placed under the transcriptional control of the CMV<sub>ie</sub> promoter by a splicing mechanism at the SD and SA sites. The plasmid pLAd.Antigen also contains a SV40 polyadenylation site, as well as prokaryotic replication origin and ampicillin-resistance gene for DNA propagation in bacteria.

**Figure 1B** illustrates another shuttle plasmid (pRAd.Cytokines) containing multiple cytokine genes such as IL-2, INF, and IL-8. The shuttle plasmid pRAd.Cytokines contains the right end of the adenoviral genome including the right long terminal repeats R-TR. Most of the E4 region (except

orf6) is replaced by the cytokine genes. Expression of cytokine genes is under the transcriptional control of the CMV<sub>ie</sub> promoter via an internal ribosomal entry site (IRES) and by a splicing mechanism at the SD and SA sites. The plasmid pAd.Cytokines also contains a bovine growth hormone (BGH) polyadenylation site, as well as a prokaryotic replication origin and ampicillin-resistance gene for DNA propagation in bacteria.

The recombinant adenoviral genome is assembled from the two shuttle plasmids, pLAd.Antigen and pRAd.Cytokines, which carries the left and right end of the adenoviral genome, respectively. The shuttle plasmids pLAd.Antigen and pRAd.Cytokines are digested with restriction enzymes such as XbaI and EcoRI, respectively.

As illustrated in **Figure 1C**, the fragments corresponding to the left end and right end of adenovirus from these two shuttle plasmids, pLAd.Antigen and pRAd.Cytokines, are isolated and ligated to the middle section of the adenoviral genome (the adenovirus backbone).

The ligated vector genome DNA is then transfected into 293HK cells that express the E1 proteins of adenovirus. In the presence of E1 proteins, the vector genome in which the E1 has been deleted can replicate and be packaged into viral particle, i.e. producing the recombinant adenoviral vector that can be used as a genetic vaccine of the present invention. The E1 region which is preserved in a native adenoviral genome but deleted from the recombinant viral genome is an example of the pathologic region native to the native progenitor of the recombinant virus: the wild type adenovirus.

**Figure 5** illustrates an example of a genetic vaccine constructed by using the method described above. The replication defective adenovirus, type 5, is the vector backbone into which viral antigen and cytokines are inserted in the E1 region. The viral antigens are expressed using the CMV<sub>ie</sub> promoter. The gene for the viral antigen is followed by the gene encoding INF- $\gamma$  and GM-CSF, utilizing 2 IRES sequences to achieve expression of the three proteins from a single mRNA. IL2 and IL4 are controlled by a second CMV<sub>ie</sub> promoter as a bi-cistronic cassette, followed by a second bi-cistronic cassette that express the two subunits of IL12 in the E4 region. Those skilled in the art will appreciate that the present invention is not limited to the structure discussed above, but that alternative cytokines may be used alone or in combination with



these and/or other cytokines. The detailed information about of these cytokines are described in the following section.

## **2. Cytokines Co-Expressed With Viral Antigens**

5           The recombinant virus of the present invention may also express an immuno-stimulator to mimic cytokine-releasing response of a host cell upon viral infection and further augment immune response to the viral antigen co-expressed from the recombinant virus. The immuno-stimulator may be an immunoenhancing cytokine to further stimulate the immune system. The  
10 recombinant virus may encode one or multiple cytokines in any combination. Alternatively, multiple cytokines may be expressed by more than one recombinant virus or delivered to the host by using other techniques such as delivery via naked DNA plasmids or injection of cytokine proteins.

          Examples of cytokine include, but are not limited to, interleukin-2,  
15 interleukin-4, interleukin-8, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF).

          Cytokines are immunomodulatory molecules particularly useful in the vaccines of the invention as they are pleiotropic mediators that modulate and  
20 shape the quality and intensity of the immune response. Cytokines are occasionally autocrines or endocrines, but are largely paracrine hormones produced in nature by lymphocytes and monocytes.

          As used herein, the term "cytokine" refers to a member of the class of proteins or peptides that are produced by cells of the immune system and that  
25 regulate or modulate an immune response. Such regulation can occur within the humoral or the cell mediated immune response and includes modulation of the effector function of T cells, B cells, NK cells, macrophages, antigen-presenting cells or other immune system cells.

          Cytokines are typically small proteins or glycoproteins having a  
30 molecular mass of less than about 30 kDa. As used herein the term cytokine encompasses those cytokines secreted by lymphocytes and other cell types (often designated as lymphokines) as well as cytokines secreted by monocytes and macrophages and other cell types (often designated as monokines). As used herein, the term cytokine encompasses those cytokines  
35 secreted by lymphocytes and other cell types as well as cytokines secreted by

monocytes and macrophages and other cell types. The term cytokine includes the interleukins, such as IL-2, IL-4, IL-5, IL-8, IL-10, IL-11, IL-12, IL-15, and IL-18, which are molecules secreted by leukocytes that primarily affect the growth and differentiation of hematopoietic and immune system cells, and human proinflammatory cytokines such as IL-1a, TNF-a and TNF-b). The term cytokine also includes hematopoietic growth factors and, in particular, colony stimulating factors such as colony stimulating factor-1, granulocyte colony stimulating factor and granulocyte macrophage colony stimulating factor.

The cytokines can have the sequence of a naturally occurring cytokine or can have an amino acid sequence with substantial amino acid sequence similarity, e.g., 60-95% amino acid sequence similarity, preferably 70-98% amino acid sequence, and most preferably 75-95% amino acid sequence similarity to the sequence of a naturally occurring cytokine.

Thus, it is understood that limited modifications to a naturally occurring sequence can be made without destroying the biological function of the cytokine. For example, minor modifications of gamma interferon that do not destroy its function fall within the definition of gamma interferon. These modifications can be deliberate, as through site-directed mutagenesis, or can be accidental such as through mutation. The preferred cytokines are IL-2, IL-8, IL-12, or  $\gamma$ -interferon,  $\beta$ -interferon,  $\lambda$ -interferon, GM-CSF, or G-CSF or a combination thereof.

Interleukin-2 is a lymphokine produced by helper T cells and is active in controlling the magnitude and type of the immune response. Smith, K. A., *Ann. Rev. Immunol.* 2, 319-333 (1984). Other functions have also been ascribed to IL-2 including the activation of NK cells (Minato, N. et al., *J. Exp. Med.* 154, 750 (1983)) and the stimulation of cell division in large granular lymphocytes and B cells. Tsudo, M. et al., *J. Exp. Med.* 160, 612-616 (1984). Studies in mice and humans have demonstrated that deficient immune responsiveness both *in vivo* and *in vitro* can be augmented by IL-2. For example, exogenous IL-2 can restore the immune response in cyclophosphamide-induced immunosuppressed mice (Merluzzi, V. J. et al. *Cancer Res.* 41, 850-853 (1981)) and athymic (nude) mice. Wagner, H. et al. *Nature* 284, 278-80 (1982). Furthermore, IL-2 can restore responsiveness of lymphocytes from patients with various immunodeficiency states such as

leprosy and cancer. Vose, B. M. et al. *Cancer Immuno.* 13, 105-111 (1984).  
The genes for murine (Yokota, T. et al. *Proc. Natl. Acad. Sci. USA* 82, 68-72  
(1985)) and human (Taniguchi, T. et al. *Nature*, 302, 305-307 (1983)) IL-2  
have been cloned and sequenced.

5 Interleukin-4 is a T cell derived factor that acts as an induction factor  
on resting B cells, as a B cell differentiation factor and as a B cell growth  
factors. Sevenusar, E. *Eur. J. Immunol.* 17, 67-72 (1987). The gene for  
human IL-4 has been isolated and sequenced. Lee, F. et al. *Proc. Natl. Acad.*  
*Sci. USA* 83, 2061-2065 (1986).

10 IL-12 is a recently characterized heterodimeric cytokine that has a  
molecular weight of 75 kDa and is composed of disulfide-bonded 40 kDa and  
35 kDa subunits. It is produced by antigen presenting cells such as  
macrophages, and binds to receptors on activated T, B and NK cells (Desai,  
B. B., et al., *J. Immunol.*, 148:3125-3132 (1992); Vogel, L. A., et al., *Int.*  
15 *Immunol.*, 8:1955-1962 (1996)). It has several effects including 1) enhanced  
proliferation of T cells and NK cells, 2) increased cytolytic activities of T cells,  
NK cells, and macrophages, 3) induction of IFN- $\gamma$  production and to a lesser  
extent, TNF- $\alpha$  and GM-CSF, and 4) activation of TH1 cells. (Trinchieri, G., et  
al., *Blood*, 84:4008-4027 (1994). IL-12 has been shown to be an important  
20 costimulator of proliferation in Th1 clones (Kennedy et al., *Eur. J. Immunol.*  
24:2271-2278 (1994)) and leads to increased production of IgG2a antibodies  
in serum (Morris, S. C., et al., *J. Immunol.* 152:1047-1056 (1994); Germann,  
T. M., et al., *Eur. J. Immunol.*, 25:823-829 (1995); Sher, A., et al., *Ann. N.Y.*  
*Acad. Sci.*, 795:202-207 (1996); Buchanan, J. M., et al., *Int. Immunol.*, 7:1519-  
25 1528 (1995); Metzger, D. W. et al., *Eur. J. Immunol.*, 27:1958-1965 (1997)).  
Administration of IL-12 can also temporarily decrease production of IgG1  
antibodies (Morris, S. C., et al., *J. Immunol.* 152:1047-1056 (1994); McKnight,  
A. J., *J. Immunol.* 152:2172-2179 (1994); Buchanan, J. M., et al., *Int.*  
*Immunol.*, 7:1519-1528 (1995)), indicating suppression of the Th2 response.  
30 The purification and cloning of IL-12 are disclosed in WO 92/05256 and WO  
90/05147, and in EP 322,827 (identified as "CLMF"). All of the above effects  
were observed in adult animals.

Interferons (IFNs) are relatively small, species-specific, single chain  
polypeptides, produced by hostian cells in response to exposure to a variety  
35 of inducers such as viruses, polypeptides, mitogens and the like. They exhibit

antiviral, antiproliferative and immunoregulatory properties and are, therefore, of great interest as therapeutic agents in the control of cancer and various other antiviral diseases (J. Desmyter et al., *Lancet* 11, 645-647 (1976); R. Derynck et al., *Nature* 287, 193 (1980)). Human interferons are grouped into three classes based on their cellular origin and antigenicity:  $\alpha$ -interferon (leukocytes),  $\beta$ -interferon (fibroblasts) and  $\gamma$ -interferon (B cells). Recombinant forms of each group have been developed and are commercially available.

$\gamma$ -interferon is also a T cell derived molecule which has profound effects on the immune response. The molecule promotes the production of immunoglobulin by activated B cells stimulated with interleukin-2.  $\gamma$ -interferon also increases the expression of histocompatibility antigens on cells which associated with viral antigens to stimulate cytotoxic T cells. The gene for human  $\gamma$ -interferon has been isolated and sequenced. Gray, P. W. et al., *Nature* 295, 503-508 (1982).

Human alpha interferons (also known as Leukocyte interferons) comprise a family of about 30 protein species, encoded by at least 14 different genes and about 16 alleles. Some of these alpha interferon protein species have been shown to have antiviral, antigrowth and immunoregulatory activities. See, e.g., Pestka et al., *Ann. Rev. Biochem.*, 56:727 (1987). The therapeutic efficacy of human alpha interferons has been established for human cancers and viral diseases. For example, recombinant interferons (IFN alpha-2a, IFN alpha-2b, IFN alpha-2c), cell-line derived interferon (IFN alpha-n1) and interferon derived from leukocytes (IFN alpha-n3) are currently used for the treatment of Condyloma acuminata, hepatitis (Weck et al., *Am. J. Med.*, 85(Suppl 2A):159 (1988); Korenman et al., *Annal. Intern. Med.*, 114:629 (1991); Friedman-Kien et al., *JAMA*, 259:533 (1988)), for the regression of some malignancies (Baron et al., *JAMA*, 266:1375 (1991)), for the treatment of AIDS related Kaposi's sarcoma (Physicians Desk Reference, 47th edit., eds. Medical Economics Data, Montvale, N.J., p. 2194 and 2006 (1993)) and are currently being considered for the treatment of human acquired immunodeficiency syndrome (AIDS) either alone or in combination with other antiviral agents (Hirsch, *Am. J. Med.*, 85(Suppl 2A):182 (1988)).

$\beta$ -interferon has been shown to be a glycoprotein by chemical measurement of its carbohydrate content. It has one N-glycosidyl attachment

site (E. Knight, Jr., *Proc. Natl. Acad. Sci.*, 73, 520 (1976); E. Knight, Jr., and D. Fahey, *J. Interferon Res.*, 2 (3), 421 (1982)). Even though not much is known about the kinds of sugars which make up the carbohydrate moiety of  $\beta$ -interferon, it has been shown that the carbohydrate moiety is not essential for its antigenicity, biological activity or hydrophobicity (T. Taniguchi et al., supra; E. Knight, Jr., supra; and E. Knight, Jr. and D. Fahey, supra). Beta-interferon can be induced in fibroblasts by viral challenge and contains about 165 amino acids. The sequence of  $\alpha$ -interferon is known. Fiers et al. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 299:29-38 (1982).

GM-CSF is a cytokine important in the maturation and function of dendritic cells. It binds receptors on dendritic cells and stimulates these cells to mature, present antigen, and prime naive T cells. Dendritic cells form a system of highly efficient antigen-presenting cells. After capturing antigen in the periphery, dendritic cells migrate to lymphoid organs and present antigens to T cells. These potent antigen-presenting cells are unique in their ability to interact with active naive T cells. The potent antigen-presenting capacity of dendritic cells may be due in part to their unique life cycle and high level expression of major histocompatibility complex class I and II molecules and co-stimulatory molecules. The sequence of human GM-CSF is known. Wong et al., *Science* 228:810-815 (1985).

Granulocyte colony stimulating factor (G-CSF) is one of the hematopoietic growth factors, also called colony stimulating factors, that stimulate committed progenitor cells to proliferate and to form colonies of differentiating blood cells. G-CSF preferentially stimulates the growth and development of neutrophils, and is useful for treating in neutropenic states. Welte et al., *PNAS-USA* 82: 1526-1530 (1985); Souza et al., *Science* 232: 61-65 (1986) and Gabilove, J. *Seminars in Hematology* 26: (2) 1-14 (1989). G-CSF increases the number of circulating granulocytes and has been reported to ameliorate infection in sepsis models. G-CSF administration also inhibits the release of tumor necrosis factor (TNF), a cytokine important to tissue injury during sepsis and rejection. See, e.g., Wendel et al., *J. Immunol.*, 149:918-924 (1992). The cDNAs for human (Nagata et al., *Nature* 319:415, 1986) and mouse G-CSF (Tsuchiya et al., *PNAS* 83, 7633, 1986) have been isolated, permitting further structural and biological characterization of G-CSF.

In humans, endogenous G-CSF is detectable in blood plasma. Jones et al., *Bailliere's Clinical Hematology* 2 (1): 83-111 (1989). G-CSF is produced by fibroblasts, macrophages, T cells trophoblasts, endothelial cells and epithelial cells and is the expression product of a single copy gene comprised of four exons and five introns located on chromosome seventeen. Transcription of this locus produces a mRNA species which is differentially processed, resulting in two forms of G-CSF mRNA, one version coding for a protein of 177 amino acids, the other coding for a protein of 174 amino acids. Nagata et al., *EMBO J* 5: 575-581 (1986). The form comprised of 174 amino acids has been found to have the greatest specific *in vivo* biological activity. G-CSF is species cross-reactive, such that when human G-CSF is administered to another host such as a mouse, Canine or monkey, sustained neutrophil leukocytosis is elicited. Moore et al. *PNAS-USA* 84: 7134-7138 (1987).

The present invention provides an effective means for enhancing the immune response to the specific foreign antigenic polypeptides of recombinant viruses. Although any foreign antigenic polypeptide can be used in the vaccine of the present invention, the vaccine is particularly useful in vaccines against the HIV virus and the Ebola virus, since these viruses have a negative effect on the host's immune system. The vaccine is also very useful for immunization against hepatitis B and C virus.

### 3. Genetic Vaccines Against HIV Infection

The genetic vaccine of the present invention also addresses the need for an efficient vaccine against the HIV virus. According to the present invention the genetic vaccine may be a recombinant benign virus in which the viral genome carries one or more antigens from HIV, such as HIV glycoproteins (e.g. GP120 and GP41) or capsid proteins (e.g. P24). Sequences of these HIV antigens may be modified such as deletion of the immunosuppressive regions of the HIV glycoproteins.

The HIV virus causes the disease known as Acquired Immune Deficiency Syndrome (AIDS). AIDS has been described as a modern plague since its first description in 1981, it has claimed over 60,000 victims, and accounted for over 32,000 deaths in the United States alone. The disease is characterized by a long asymptomatic period followed by a progressive

degeneration of the immune system and the central nervous system. The virus may remain latent in infected individuals for five or more years before symptoms appear, and thus, the true impact of the disease has yet to be felt. Many Americans may unknowingly be infected and capable of infecting others who might come into contact with their body fluids. Thus, if unchecked, the personal, social and economic impact of AIDS will be enormous.

The HIV virus is a retrovirus. Thus, its genetic material is RNA, which encodes the information for viral replication. Upon infection of a host cell, the RNA acts as a template for the transcription to DNA, which is catalyzed by an enzyme called reverse transcriptase. The DNA so produced enters the cell nucleus where it is integrated into the host DNA as a provirus. When properly activated, the retroviral-derived DNA is transcribed and translated to produce RNA containing virions, which are then released from the cell by a budding process.

When an individual becomes infected with HIV, the virus preferentially attaches to and enters a particular class of white blood cells, called T4 lymphocytes, which are characterized by the presence of a cell surface marker termed CD4. These white blood cells play an integral role in the immune system, functioning as critical components of both the humoral and cellular immune response. Much of the deleterious effect of HIV can be attributed to the functional depression or destruction of T4 lymphocytes.

The intact HIV virion is roughly spherical and is approximately 110 nm in diameter. The virion has an outer membrane covered with spike-like structures made up of glycoprotein, gp160/120. In addition, there exists a transmembrane protein termed gp41. Inside the virion are two structural proteins: an outer shell composed of the phosphoprotein, p17, and an inner nucleoid or central core made up of the phosphoprotein, p24. The viral RNA is present inside the core along with two copies of the reverse transcriptase enzyme, p66/51, which is necessary for the synthesis of viral DNA from the RNA template. The HIV RNA genome encodes three major structural genes: gag, pol and env, which are flanked at either end by long terminal repeat (LTR) sequences. The gag gene codes for the group-specific core proteins, p55, p39, p24, p17 and p15. The pol genes code for the reverse transcriptase, p66/p51, and the protease, p31. The env genes encode the outer envelope glycoprotein, gp120, and its precursor, gp160, and the

transmembrane glycoprotein, gp41. Some of the genes tend to be highly variable, particularly the env genes. In addition, there are five other genes, not shared by other retroviruses, which are either involved in transcriptional or translational regulation or encode other structural proteins. The entire HIV genome has now been sequenced. See Ratner et al. Nature 313:277 (1985), which is incorporated herein by reference.

The HIV envelope protein has been extensively described, and the amino acid and RNA sequences encoding HIV envelope from a number of HIV strains are known. See Myers, G. et al., *Human Retroviruses and AIDS: A compilation and analysis of nucleic acid and amino acid sequences*, Los Alamos National Laboratory, Los Alamos, N.M. (1992). The env genes of various strains of HIV are predicted to encode proteins of 850 to 880 amino acids. Extensive glycosylation of the Env precursor polyprotein during synthesis produces gp160 (about 160 kilodaltons) which is also the major form of the env gene product detected in infected cells. Gp160 forms a homotrimers and undergoes glycosylation with the Golgi apparatus.

The functional domains of gp160 includes, starting from N-terminus, Signal peptide, Variable regions 1 through 5 which encompass CD4 binding sites (e.g., Thr<sup>257</sup>, Trp<sup>427</sup>, Asp<sup>368</sup>/Glu<sup>370</sup>, and Asp<sup>457</sup>), Proteolytic processing site (also called the cleavage site between gp120 and gp41), Fusion domain, Leucine zipper motif, transmembrane domain, and Lentivirus lytic peptides (LLP) 1 and 2. Although the nucleotide and amino acid sequences of gp120 and the numbering thereof from various isolates and strains of HIV may differ, the region encoding the functional domains can be readily identified by the teaching in Luciw (1996) in "Fundamental Virology", 3<sup>rd</sup> ed., eds., Fields et al., Lippincott-Raven Publishers, Philadelphia, Chapter 27, pp. 845-916.

The signal peptide at the N-terminus of the Env precursor gp160 directs ribosomes translating the nascent protein to the endoplasmic reticulum; an intracellular proteinase removes this signal peptide during Env gp biogenesis. The Env precursor gp160 is cleaved at the processing site by a cellular protease to produce gp120 (designated SU subunit) and gp41 (designated TM subunit). Gp120 contains most of the external, surface-exposed, domains of the envelope glycoprotein complex. Gp41 contains a transmembrane domain and remains in a trimeric configuration, and it interacts with gp120 in a non-covalent manner. The subunits of gp41 include:



Fusion peptide, Leucine zipper-like region, transmembrane domain (TM), LLP1 and LLP2.

5 The gp120 subunit contains five variable regions and six conserved regions. The variable (V) domains and conserved (C) domains of gp120 are specified according to the nomenclature of Modrow et al. (1987) "Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: predictions of antigenic epitopes in conserved and variable regions", J. Virol. 61:570-578.

10 The gp120 molecule consists of a polypeptide core of 60,000 daltons, which is extensively modified by N-linked glycosylation to increase the apparent molecular weight of the molecule to 120,000 daltons. The positions of the 18 cysteine residues in the gp120 primary sequence, and the positions of 13 of the approximately 24 N-linked glycosylation sites in the gp120 sequence are common to all gp120 sequences. The hypervariable domains  
15 contain extensive amino acid substitutions, insertions and deletions. Sequence variations in these domains result in up to 30% overall sequence variability between gp120 molecules from the various viral isolates. Despite this variation, all gp120 sequences preserve the virus's ability to bind to the viral receptor CD4 and to interact with gp41 to induce fusion of the viral and  
20 host cell membranes.

The HIV virus attaches to host cells by an interaction of the envelope glycoproteins with a cell surface receptor. It appears that when HIV makes contact with a T4 cell, gp120 interacts with the CD4 receptor. Recently, the crystal structure of the core domain of HIV-1 gp120 (strain HXB-2, a clade B virus) has been solved by complexing the protein with a fragment of human  
25 CD and an antigen-binding fragment from a virus-neutralizing antibody that blocks chemokine-receptor binding. Kwong et al. (1998) "Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody", Nature 393:648-659. These studies revealed that the gp120 core has a unique molecular structure that comprises two  
30 domains—an "inner domain" (which faces gp41) and an "outer" domain (which is mostly exposed on the surface of the oligomeric envelope glycoprotein complex). The two gp120 domains are separated by a "bridging sheet" that is not part of either domain. Binding to CD4 causes a conformational change in  
35 gp120 which exposes the bridging sheet and may move the inner and outer

domains relative to each other. It was also found that most of the carbohydrate molecules which are added to gp120 are added to the outer domain. This is consistent with the idea that that virus uses carbohydrate molecules to mask external antigenic epitopes on gp120.

5           Gp120 not only binds to the cellular CD4 receptor but also to HIV coreceptors such as the cellular chemokine receptors (e.g. CCR5). Upon binding to the receptor and/or coreceptor, the viral envelope is then fused with the cell membrane and the inner core of the virus enters the infected cell where the transcription of RNA into a DNA provirus is catalyzed by reverse transcriptase. The provirus may remain in the cell in a latent form for some months or years, during which time the infected individual is asymptomatic. However, if the virus is later activated causing viral replication and immuno-suppression the individual will than be susceptible to the opportunistic infections associated with AIDS.

10           In one embodiment of the HIV vaccine of the present invention, a recombinant virus is provided for eliciting strong immune response against infection of HIV. The recombinant virus comprises: an antigen sequence heterologous to the recombinant virus that encodes an antigen from human immunodeficiency virus (HIV), expression of the HIV antigen eliciting an immune response directed against the HIV antigen and cells expressing the HIV antigen in a host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the HIV antigen. In a preferred embodiment, the recombinant virus is replication-incompetent and does not cause a malignancy naturally associated with HIV in the host. The recombinant virus is used as a genetic vaccine to be administered to a host to induce or elicit strong and long-lasting immunity against HIV infection.

15           In comparison with other approaches for developing HIV vaccine using denatured or attenuated HIV virion, the approach of the present invention should be safer and more efficient in eliciting strong immune response but not creating risks of reactivation of HIV, probably through recombination with the wild type HIV infecting the host.

20           According to the present invention, the HIV antigen expressed by the genetic vaccine may be any antigen derived from a HIV virus, such as HIV

surface, core/capsid, regulatory, enzyme and accessory proteins. Examples of HIV surface protein include, but are limited to the products of the *env* gene such as gp120 and gp41. Examples of HIV capsid protein include, but are limited to the products of the *gag* gene such as the cleavage products of the Pr55<sup>gag</sup> by the viral encoded protease PR: the mature capsid proteins MA (p17), CA (p24), p2, NC (p7), p1 and p6. Herderson et al. (1992) J. Virol. 66:1856-1865. Examples of viral regulatory proteins include, but are not limited to the products of the *tat* and *rev* genes: Tat and Rev. Examples of viral enzyme proteins include, but are not limited to the products of the *pol* gene: p11 (protease or PR), p51 (reverse transcriptase or RT), and p32 (integrase or IN). Examples of viral accessory proteins include, but are not limited to the products of the *vif*, *vpr*, *vpx*, *vpu* and *nef* genes: Vif, Vpr, Vpx, Vpu and Nef.

In one embodiment, HIV Nef protein may serve as the HIV antigen expressed by the recombinant virus of the present invention. For example, sequence encoding Nef (e.g., the *nef* sequence at position 8152-8523 for BH10 strain of HIV and at position 8787-9407 for pNL4-3 strain of HIV) may be inserted into the vector.

In another embodiment, HIV Rev protein may serve as the HIV antigen expressed by the recombinant virus of the present invention. For example, sequence encoding Rev (e.g., the *rev1* sequence at position 5969-6044 and the *rev2* sequence at position 8369-8643 for pNL4-3 strain of HIV) may be inserted into the vector.

In yet another embodiment, full length HIV Gag protein may serve as the HIV antigen expressed by the recombinant virus of the present invention. For example, sequence encoding full length Gag (e.g., the *gag* sequence at position 112-1650 for BH10 strain of HIV and at position 790-2292 for pNL4-3 strain of HIV) may be inserted into the vector.

Alternatively, capsid protein from HIV Gag protein (e.g. p24 CA) may serve as the HIV antigen expressed by the recombinant virus of the present invention. For example, sequence encoding p24CA (e.g., the sequence at position 1186-1878 for BH10 strain of HIV and at position 508-1200 for pNL4-3 strain of HIV) may be inserted into the vector.

In yet another embodiment, the HIV antigen expressed by the recombinant virus is derived from the *env* gene products. For example, the antigen is derived from the Env protein.

According to the embodiment, modifications or mutagenesis may be used to delete or mutate in certain region(s) of Env to render it non-functional and yet still contains neutralizing epitopes for its natural genicity. For example, the proteolytic processing site of Env may be deleted or mutated to render it resistant to cleavage by cellular protease to produce gp120 and gp41 fragments. Deletion or mutation may also be carried out on the transmembrane and cytoplasmic domains of gp41 such as the TM, LLP-1 and LLP-2 domains. Compared to the wild type Env, the mutated Env protein should have a reduced risk of being incorporated into a wild type HIV that infects the host and being exploited by HIV in its furtherance of the goal: destruction the host's immune system.

For example, wildtype HIV Env can be modified in the following ways. Wildtype gp120 sequence from BH10 strain of HIV and containing Env, Tat, and Rev coding sequences can be digested with restriction enzymes EcoR I and Xho I to produce a fragment starting from nucleotide 5101 and ending at nucleotide 8252. The cytosolic domain of Env can be removed by deleting nucleotides from the coding sequence at position 7848-8150 for BH10 strain, and 8610-8785 for pNL4-3 strain of HIV. The cleavage site of Env can be removed by deleting 12 nucleotides encoding amino acid sequence REKR at position 7101-7112 for BH10 strain, and 7736-7747 for pNL4-3 strain of HIV.

Also according to this embodiment, the modified Env protein may contain deletions in the regions that do not contain neutralizing epitopes. For example, the V1 and V5 domains of gp120 may be deleted without sacrificing the natural antigenicity of gp120. Portions of the V2 and V3 domains of gp120 that do not contain neutralizing epitopes may also be deleted. Although the principle neutralizing domain (PND) has been found in the V3 domain, V2 and C4 domains of gp120 have also been found to contain neutralizing epitopes. Among various strains or clades of HIV, the amino acid sequences of the neutralizing epitopes may be variable. However, it has been found that the amount of variation is highly constrained. Thus, the sequences not containing the neutralizing epitopes should be readily determined.

For example, sequence encoding V1 region of Env can be deleted at position 5961-6032 for BH10 strain, and 6602-6673 for pNL4-3 strain of HIV. Sequence encoding V2 region of Env can be deleted at position 6060-6161 for BH10 strain, and 6700-6796 for pNL4-3 strain of HIV. Optionally,  
5 sequence encoding both V1 and V2 regions of Env can be deleted at position 5961-6161 for BH10 strain, and 6602-6796 for pNL4-3 strain of HIV.

Alternatively, the HIV antigen expressed by the recombinant virus may be a subunit of gp120 which contains one or more selected variable (V) and/or conserved (C) domains. For example, the HIV antigen may be a gp120  
10 subunit containing V2, V3 and C4 domains, or V3 and C4 domains. The location of neutralizing epitopes in the V3 domain is well known. It has been found that neutralizing epitopes in the V2 and C4 domains are located between residues 163 and 200 and between about 420 and 440, respectively. In addition, residues for antibody binding also include residues 171, 174, 177,  
15 181, 183, 187, 188 in the V2 domain and residues 429 and 432 in the C4 domains. Berman et al. (1999) Virology 265:1-9; and Berman (1998) AIDS Res. Human Retroviruses 15:115-132.

In another embodiment, the HIV antigen expressed by the recombinant virus of the present invention may be a modified Env protein that contains  
20 deletions and/or mutations in the glycosylation sites. The gp120 of HIV-1 contains 24 potential sites for N-linked glycosylation (Asn-X-Ser/Thr); about 13 of the 24 glycosylation motifs are conserved in the different viral isolates. Analysis of HIV-1 Env gp proteins has demonstrated that 17 of 24 potential glycosylation sites are modified with carbohydrate side chains. Mizuochi et al.  
25 (1990) J. Biol. Chem. 265:8519-8524; and Leonard et al. (1990) J. Biol. Chem. 265:10373-10382. Because of the extensive glycosylation of Env gp proteins, very few regions of the peptide backbone of gp120 protrude from the carbohydrate mass. Some of the glycosylation sites have been found in non-neutralizing epitopes that dilute the immunity against true neutralizing epitopes  
30 or serve as decoy epitopes. Thus, deletion or mutation of these glycosylation sites may enhance immunity of the antigen by unmasking the true neutralizing epitopes.

In another embodiment, the different HIV antigens may be expressed by the same recombinant virus of the present invention. For example, both  
35 Env, Tat and Rev proteins may be expressed from the same promoter such as

a CMV early promoter via a retroviral splicing donor-acceptor mechanism. Optionally, HIV Gag protein, either in full length or a truncated or modified form (e.g., capsid protein p24), may also be expressed together with other HIV antigens such as Env, Tat and Rev. Further, these HIV antigens may be expressed together with the immuno-stimulator(s) (e.g., IL-2, IL-12, INF- $\gamma$ , and GMCSF) in single or multiple copies by the same recombinant viral vector.

For example, the sequences encoding the HIV antigens may be inserted into E1 region of an adenoviral vector and expressed from a CMV early promoter via a retroviral splicing donor-acceptor mechanism or an IRES mechanism. The sequences encoding the immuno-stimulators may be inserted into E4 region of the same adenoviral vector and expressed from another CMV early promoter via a retroviral splicing donor-acceptor mechanism or an IRES mechanism.

In yet another embodiment, the sequence encoding the HIV antigen in the recombinant virus of the present invention is a mosaic antigen that contains sequences from different strains, isolates and/or clades of HIV viruses. A strain of HIV is the HIV isolated from an individual (an isolate), characterized and given a strain name (e.g., MN, LAI). Because of the heterogeneity of HIV, not two isolates are exactly the same. A group of related HIV isolates are classified according to their degree of genetic similarity such as of their envelop proteins. There are currently two groups of HIV-1 isolates, M and O. The M group consists of at least 9 clades (also called subtypes), A through I. The O group may consist of a similar number of clades. Clades are genetically distinct but are all infectious. It is believed that by using a mosaic HIV antigen in the design of the genetic vaccine of the present invention the vaccine produced should have an enhanced ability to stimulate the production of anti-HIV antibodies and HIV-specific cytotoxic T lymphocytes (CTLs) against a wider spectrum of "wild type" HIV strains.

In one embodiment, the mosaic HIV antigen in the recombinant virus contains antigens from multiple clades of HIV-1, including clade A (Accession No: HIV-1 92UG037WHO.0108HED), B (Accession No: pNL4-3), C (Accession No: HIV-1 92BR025WHO.109HED), D (Accession No: HIV-1 92UG024.2), E (Accession No: HIV-1 93TH976.17), F (Accession No: HIV-1 93BR020.17), and G (Accession No: HIV-1 92RU131.9). Optionally, multiple repeats of restriction fragments of HIV antigen (e.g., Ava I fragments) from

different clades may be linked head-to-tail to generate an even more complex mosaic HIV antigen.

For example, an adenoviral vector may be constructed to the V3 loops of multiple clades as the mosaic HIV antigen. Optionally, HIV antigens with gp41 deletion from multiple clades may serve as the mosaic HIV antigen. Alternatively, HIV antigens from multiple clades with V1 and V2 loops deleted from clade B (pNL4-3) may serve as the mosaic HIV antigen.

Yet optionally, a human gene Thy-1 GPA anchor sequence encoding amino acid sequence SWLLLLLSLSLLQATDFMSL [SEQ ID NO: 9] may be added to the recombinant viral construct.

In another embodiment, the mosaic HIV antigen contains an Env protein which comprises variable and constant domains of gp120 derived from different strains, isolates and/or clades of HIV viruses. For example, V2 domain from clade B of the M group may be mixed with V3 and C4 domains from clade C of the O group to generate a mosaic HIV antigen. Vaccination of individuals with such a mosaic antigen may stimulate CTLs with cross-clade activity. In another word, these CTLs can recognize and kill target cells infected HIV from different clades.

Alternatively, the recombinant virus may express a plurality of HIV antigens, each of which is an antigen from a different strain, isolate or clade of HIV. For example, *env* genes from different clades of HIV can be cloned into the recombinant virus and expressed in tandem to produces various Env proteins from these clades in the host cells. It is believed that expressing various Env proteins from different strains, isolates or clades of HIV in the host cells should enhance the ability of the genetic vaccine of the present invention to stimulate the production of anti-HIV antibodies and HIV-specific cytotoxic T lymphocytes (CTLs) against a wider spectrum of "wild type" HIV strains. The host vaccinated with such a vaccine would be able to be immunized from infection of various strains of HIV.

By using the genetic vaccine of the present invention, individuals not infected by HIV may be immunized against HIV. For HIV-infected individuals the vaccine may also be used boost their immune response and help fight against this virulent virus. Since the genetic vaccine can express high level of antigens and/or a variety of HIV glycoproteins and capsid proteins simultaneously, the vaccinated individuals should be immunized against

various strains of HIV, such as HIV-1 and HIV-2. Additionally, since the genetic vaccine can express high levels of cytokines to mimic the body's response to natural viral infection, the body's immune response to such a genetic vaccine against HIV should be strong and long-lasting, thereby achieving a life-long immunity against this deadly virus.

#### 4. Genetic Vaccines Against Hepatitis Viruses

The genetic vaccine of the present invention also addresses the need for an efficient vaccine against hepatitis viruses such as hepatitis A, B, C, D, and E viruses. According to the present invention the genetic vaccine may be a recombinant benign virus in which the viral genome carries one or more antigens from a hepatitis virus, such as glycoproteins and core proteins of the hepatitis virus. Sequences of these HIV antigens may be modified such as deletion of the pathogenic regions of the hepatitis glycoproteins or coreproteins.

In particular, the recombinant virus of the present invention can be used as a vaccine to immunize individuals against Hepatitis B infections. Viral hepatitis B is caused by the Hepatitis B virus (HBV). HBV is estimated to have infected 400 million people throughout the world, making HBV one of the most common human pathogens. Hepatocellular carcinomas (HCC), one of the most common cancers afflicting humans, is primarily caused by chronic HBV infection.

HBV is a mostly double-stranded DNA virus in the *Hepadnaviridae* family. The HBV genome is unique in the world of viruses due to its compact form, use of overlapping reading frames, and dependence on a reverse-transcriptase step, though the virion contains primarily DNA. The HBV genome has four genes: pol, env, pre-core and X that respectively encode the viral DNA polymerase, envelope protein, pre-core protein (which is processed to viral capsid) and protein X. The function of protein X is not clear but it may be involved in the activation of host cell genes and the development of cancer.

The diagnosis of HBV infection is generally made on the basis of serology. Virtually all individuals infected with HBV will have detectable serum hepatitis surface antigens (HBsAg). Despite notable successes of vaccines against HBV infection, it is still an on-going task. A review on modern hepatitis



vaccines, including a number of key references, may be found in the Eddleston, *The Lancet*, p. 1142, May 12, 1990. See also *Viral Hepatitis and Liver Disease*, Vyas, B. N., Dienstag, J. L., and Hoofnagle, J. H., eds., Grune and Stratton, Inc. (1984) and *Viral Hepatitis and Liver Disease*, Proceedings of the 1990 International Symposium, eds F. B. Hollinger, S. M. Lemon and H. Margolis, published by Williams and Wilkins. According to the present invention, the viral antigen may be a surface antigen or core protein of hepatitis B virus such as the small hepatitis B surface antigen (SHBsAg) (also referred to as the Australia antigen), the middle hepatitis B surface antigen (MHBsAg) and the large hepatitis B surface antigen (LHBsAg).

Antigens of different types of HBV, such as Asian type C and America type A, may be expressed by the recombinant virus to elicit immune response to these types of HBV. The HBV surface antigen (HBsAg) or the core antigen (HBcAg) may be expressed by the recombinant virus of the present invention, separately or in combination (HBsAg + HBcAg).

For example, the sequences encoding multiple HBV antigens may be inserted into E1 or E4 region of an adenoviral vector and expressed from a CMV early promoter via a retroviral splicing donor-acceptor mechanism or an IRES mechanism. Further, these HBV antigens may be expressed in combination with one or more immuno-stimulators such as IL-2, IFN- $\gamma$  and GMCSF in single or multiple copies. Sequences encoding these cytokines may be inserted into E4 or E1 region that is not occupied by the antigen sequences and expressed from another CMV early promoter via a retroviral splicing donor-acceptor mechanism or an IRES mechanism.

Specific combinations of inserts include, but are not limited to, HBsAg + HBcAg; HBsAg + HBcAg + IL-2; HBsAg + HBcAg + IFN- $\gamma$  + GMCSF; and HBsAg + IFN- $\gamma$  + IFN- $\gamma$  + GMCSF.

The sequences encoding the immuno-stimulators may be inserted into E4 region of the same adenoviral vector and expressed from another CMV early promoter via a retroviral splicing donor-acceptor mechanism or an IRES mechanism.

Also according to the present invention, the viral antigen may be a surface antigen or core protein of hepatitis C virus such as NS3, NS4 and NS5 antigens.

For example, sequence(s) encoding the HCV antigen(s) may be inserted into E1 or E4 region of an adenoviral vector and expressed separately or in combination with one or more immuno-stimulators such as IL-2, IL-12, IFN- $\gamma$  and GMCSF in single or multiple copies.

- 5           Specific combinations include, but are not limited to,
- (1) HCV wildtype E2 + wildtype E1;
  - (2) core of HCV;
  - (3) HCV E2 + E1 + core;
  - (4) HCV E2 + E1 + core + IL-2;
  - 10       (5) HCV E2 + E1 + core + IL-2 + IFN- $\gamma$  + GMCSF; and
  - (6) HCV E2 + E1 + core + IL-2 + IFN- $\gamma$  + IL-12.

In another embodiment, multi copies of hypervariable regions (HVR) of HCV E1 and E2, e.g., five copies of HVR (5xHVR), may serve as the viral antigen in the recombinant virus, and may be expressed alone or in combination with one or more immuno-stimulators such as IL-2, IL-12, IFN- $\gamma$  and GMCSF in single or multiple copies.

- 15           Specific combinations include, but are not limited to,
- (1) E2-5xHVR + E1;
  - 20       (2) E2-5xHVR + E1 + IL-2;
  - (3) E2-5xHVR + E1 + core + IL-2;
  - (4) E2-5xHVR + E1 + core + IL-2 + IFN- $\gamma$  + GMCSF; and
  - (5) E2-5xHVR + E1 + core + IL-2 + IL-12.

25           By using the genetic vaccine of the present invention, non-hepatitis-infected individuals may be immunized against hepatitis virus. For hepatitis virus-infected individuals the vaccine may also be used boost their immune response and help fight against the hepatitis virus. Since the genetic vaccine can express high level of antigens and/or a variety of hepatitis glycoproteins and coreproteins simultaneously, the vaccinated individuals should be immunized against various strains and/or types of hepatitis virus, such as hepatitis A, B, C, D, and E virus. Additionally, since the genetic vaccine can express high levels of cytokines to mimic the body's response to natural viral infection, the body's immune response to such a genetic vaccine against

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hepatitis should be strong and long-lasting, thereby achieving a life-long immunity against the hepatitis virus.

#### 5. Genetic Vaccines Against Ebola Virus

5 The genetic vaccine of the present invention also addresses the need for an efficient vaccine against the deadly virus, Ebola virus. According to the present invention the genetic vaccine may be a recombinant benign virus in which the viral genome carries one or more antigens from Ebola hepatitis, such as glycoproteins (e.g. GP1 and GP2) of Ebola virus. Sequences of these  
10 Ebola antigens may be modified such as deletion of the immunosuppressive regions and/or other pathogenic regions of the Ebola virus.

Ebola virus is one of the most lethal viruses known to mankind with a mortality rate of up to 90%. Johnson, K.M., *Ann Intern Med* 91(1):117-9 (1979). Victims of Ebola virus infection are subjected to a horrible  
15 hemorrhagic diseases which kills in a matter of days. The natural reservoir of the virus remains unknown, as do the specifics of pathogenesis of the infection. The virus has a very specific tropism for liver cells and cells of the reticuloendothelial system, such as macrophages. Massive destruction of the liver is hallmark feature of the disease.

20 Although Ebola virus infection is rare, there is concern by public health officials about the potential for the disease to become an international epidemic as the Ebola virus is easily transmitted through human contact and is extremely contagious. Outbreaks like those that have recently occurred in Africa could happen in industrialized countries due to the rapid and extensive  
25 nature of modern travel. Recent cases of Ebola virus infection in Africa send strong warnings to be prepared for the outbreaks of this extremely dangerous infectious disease. In addition, Ebola virus has a terrifying potential if used as a biological weapon by terrorist nations or organizations. As in most cases of viral infection, the best approach to prevent an outbreak of Ebola virus is  
30 through vaccination. However, currently there is no effective vaccine nor treatment available against Ebola virus infection.

Ebola viruses are enveloped, negative strand RNA viruses, which belong to the family Filoviridae. There are three strains of filoviruses: Ebola, Marburg and Reston. The Ebola virus can enter the body a number of  
35 different ways such as an opening through which air is taken in because the

virus can travel on airborne particles and it can also enter the body through any opening in the skin, such as cuts.

The Ebola virus has a non-segmented RNA genome that encodes all the viral structural proteins (nucleoprotein, matrix proteins VP24 and VP40), non-structural proteins (VP30, VP35) and viral polymerase. Peters, C.J., West  
5 *J Med* 164(1):36-8 (1996). Among the viral proteins, the envelope glycoproteins (GP) exist in two forms, a secreted glycoprotein (50-70 kDa) and a transmembrane glycoprotein (130-170 kDa) generated by transcriptional editing. Sanchez, A. et al., *Proc Natl Acad Sci U.S.A.*, 93(8):3602-7 (1996).  
10 Although the two forms of GP share 295 amino acid homology, they have distinct binding specificities, suggesting that they play different roles in the course of viral infection. The secreted glycoprotein (sGP) is the predominant form synthesized and secreted by the infected cells. It may play a role in suppressing the host immune system (Yang, Z., et al., *Science*  
15 279(5353):1034-7 (1998)) and may serve as a decoy to allow the virus particle to escape from neutralizing antibodies, since the two forms of GPs partly share their antigenicity. Analysis of monoclonal antibodies from the human survivors of Ebola virus Zaire infection has revealed that the vast majority of them were specific to the sGP, and only a few bound weakly to GP.  
20 Maruyama, T., et al., *J Infect Dis*, 179 Suppl 1:S235-9 (1999), Maruyama, T., et al., *J Virol*, 73(7):6024-30 (1999). Although the exact mechanism by which the sGP may suppress the immune system is not clearly understood, the large amounts of sGP synthesized in the early phase of the infection are probably responsible for the inhibition of neutrophil infiltration of the infected sites  
25 (Yang, Z., et al., *Science* 279(5353):1034-7 (1998)) and the absence of humoral immune response in Ebola virus infected patients. Baize, S., et al., *Nat Med*, 5(4):423-6 (1999). This protein may also act to over-activate many types of immune cells which can lead to massive intravascular apoptosis - essentially a shut-down of the immune system. Baize, S., et al., *Nat Med*,  
30 5(4):423-6 (1999). The importance of the sGP to the Ebola virus life-cycle is also suggested by the fact it is present in all Ebola virus strains examined to date. Feldmann, H., et al., *Arch Virol Suppl*, 15:159-69 (1999).

The membrane glycoproteins are responsible for the attachment and penetration of the virions into target cells by mediating receptor binding and  
35 viral-cellular membrane fusion. Wool-Lewis, et al., *J. Virol*, 72(4):3155-60

(1998), Ito H., et al., *J. Virol*, 73(10):8907-12 (1999). They are synthesized as a single peptide precursor and cleaved by cellular enzymes (furin or cathepsin B) into the two mature forms, GP1 and GP2. The two GPs remain associated through a disulfide bond linkage and remain anchored in the viral membrane by a transmembrane (TM) domain. Ito H., et al., *J. Virol*, 73(10):8907-12 (1999); Malashkevich, V.N., et al., *Proc Natl Acad Sci U.S.A.*, 96(6):2662-7 (1999). The proteolytic cleavage site is composed of 4-5 basic amino acid residues that are similar to those found in the GPs of retrovirus, influenza, and paramyxoviruses. Garten, W., et al., *Biochimie*, 76(3-4):217-25 (1994). The cleavage event is essential for viral infectivity and is likely carried out by the same enzymes that cleave GPs of retrovirus or influenza viruses. Garten, W., et al., *Biochimie*, 76(3-4):217-25 (1994); Volchkov, V.E., et al., *Virology*, 245(1):110-9 (1994). In addition, Ebola virus GP may share a common mechanism of mediating viral infection with retroviral and influenza glycoproteins. Weissenhorn, W., et al., *Mol Membr Biol*, 16(1):3-9 (1999). Because membrane-bound GPs play critical roles in initiating virus infection and are also the predominant proteins exposed on the surface of the virions, they are the primary targets for neutralizing antibodies against the virus.

One of the properties of Ebola viruses that make them lethal to the host is their ability to suppress the host immune system. Serologic analysis of patients who died of the Ebola virus infection showed no signs of humoral or cellular immune responses. Baize, S., et al., *Nat Med*, 5(4):423-6 (1999). In contrast, antibodies against viral proteins and virus-specific T-cell activities were detected in a few survivors. Baize, S., et al., *Nat Med*, 5(4):423-6 (1999). Although the immunosuppressive mechanisms are yet to be understood, it is probable that the high levels of sGP and the immunosuppressive peptide in the GP are to blame for the absence of humoral and cellular immune responses in Ebola virus-infected patients.

The proteins that are responsible for the initial infection of Ebola virus are the viral glycoproteins. Therefore, they are the target for neutralizing antibodies. However, Ebola virus has evolved "tricks" to prevent or delay the host immune response until it is too late to recover from the infection. Conventional approaches in producing vaccines against Ebola virus are likely to be ineffective for the following reasons: (1) viral glycoproteins produced in bacteria, yeast or insect cells are not properly glycosylated and therefore do

not have the true antigenicity of the viral proteins; (2) Ebola virus is too dangerous to be produced in large amounts as an inactivated-virus vaccine; and (3) procedures of inactivating the virus often destroy the conformation of the proteins, and therefore alter their antigenicity.

5           A preferred embodiment of the present invention is a recombinant viral vaccine having nucleic acids encoding one or more antigens of Ebola virus. Restriction maps and full sequence information of the Ebola virus, including the Zaire strain, is available through GenBank.

10           The genetic vaccine is a recombinant benign virus which is replication defective or incompetent and therefore is incapable of spreading beyond initially infected cells. For example, a recombinant adenoviral vaccine of the present invention mediates high levels of Ebola viral antigen expression for a period of two or more weeks, even though Ebola viral proteins have no functional relevance to recombinant virus function.

15           In another embodiment of the invention, the recombinant virus expresses one or more modified Ebola virus antigens. The modified Ebola virus antigens are preferably Ebola virus envelope glycoproteins and/or immunologically active parts thereof. Preferably the glycoproteins are modified GP and sGP glycoproteins. The Ebola virus GP and sGP glycoproteins are  
20           modified to destroy their pathogenic and immunosuppressive functions, but retain most of their natural antigenicity, since they are expressed, folded, glycosylated, and targeted to the cellular membrane inside the cells that can be productively infected by the Ebola virus. The modifications are carried out using standard molecular genetic manipulation techniques such as restriction  
25           digests and polymerase chain reaction.

          A preferred modification of the Ebola virus envelope glycoprotein destroys the infective function of the Ebola virus GP. Any modification that destroys the infective function of Ebola virus can be used, but preferably the modification is a five amino acid deletion in the cleavage site of the GP. See  
30           Example 1. This cleavage site is composed of five basic amino acid residues, RRTRR, at position 501 from the start of the open reading frame. This deletion may be introduced into the Ebola virus GP cDNA using PCR amplification, which is performed by methods well known in the art.

          Another preferred modification of the Ebola virus viral genome  
35           prevents synthesis of the sGP. Any modification that prevents synthesis may

be employed. Preferably the modification is directed to altering the RNA editing site from UUUUUUUU to UUCUUCUU. See example 1.

Another preferred modification to Ebola virus antigen used in the present vaccines is immunosuppressive (IS) peptide located in GP2. The IS peptide motif is located at amino acids 585-609. A ten amino acid deletion between amino acid 590-600 removes its function. Second, each half of the IS peptide motif is reversed and duplicated. See **Figure 2**. This further ensures that its function has been destroyed and also increases its antigenicity.

Further it is readily apparent to those skilled in the art that variations or derivatives of the nucleotide sequences encoding Ebola virus antigen(s) of the present invention can be produced, which alter the amino acid sequence of the encoded protein. The altered expressed antigen(s) may have an altered amino acid sequence, yet still elicit immuneresponses that react with Ebola virus antigen(s), and are considered functional equivalents. In addition, fragments of the full-length genes that encode portions of the full-length protein may also be constructed. These fragments may encode a protein or peptide which elicits antibodies which react with Ebola virus antigen(s), and are considered functional equivalents.

Vaccination of an individual with the vaccines of the present invention results in entrance of adenoviral particles into cells and expression of Ebola virus antigen(s), such as the envelope glycoproteins, and the immune-stimulating cytokines. The expression of Ebola virus antigen(s) in cells induces strong and persistent immune responses as if an infection has occurred. The genetic vaccine has all of the immunogenicity of a natural infection, including expression of the natural viral proteins and long-lasting antigen stimulation, but does not have the pathogenicity of a true viral infection. In the vaccines of the present invention, the immunosuppressive mechanisms of Ebola virus are disabled, the antigens occur in their natural forms and are associated with the cell membrane, and immune stimulation lasts for weeks. The effects of this novel vaccine are long lasting and provide high rates of protection against Ebola virus infection.

The present invention is also directed to a method of immunizing a human against Ebola virus infection comprising administering the vaccines

described above. The techniques for administering these vaccines to humans are known to those skilled in the health fields.

By using the genetic vaccine of the present invention, individuals may be immunized against Ebola virus. Since the genetic vaccine can express  
5 high levels of antigens and/or a variety of glycoproteins simultaneously, the vaccinated individuals should be immunized against various strains Ebola virus. Additionally, since the genetic vaccine can express high levels of  
cytokines to mimic the body's response to natural viral infection, the body's immune response to such a genetic vaccine against Ebola virus should be  
10 strong and long-lasting, thereby achieving a life-long immunity against the Ebola virus.

#### **6. Formulation and Routes of Administration**

The present invention also relates to a pharmaceutical composition  
15 comprising the vaccine(s) described above, and a pharmaceutically acceptable diluent, carrier, or excipient carrier. Additionally the vaccine may also contain an aqueous medium or a water containing suspension, often mixed with other constituents in order to increase the activity and/or the shelf life. These constituents may be salt, pH buffers, stabilizers (such as skimmed  
20 milk or casein hydrolysate), emulsifiers, and preservatives.

An adjuvant may be included in the pharmaceutical composition to augment the immune response to the viral antigen expressed from the recombinant virus. Examples of the adjuvant include, but are not limited to, muramyl dipeptide, aluminum hydroxide, saponin, polyanions, amphipathic  
25 substances, bacillus Calmette-Guerin (BCG), endotoxin lipopolysaccharides, keyhole limpet hemocyanin (GKLH), interleukin-2 (IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and cytoxan, a chemotherapeutic agent which is believed to reduce tumor-induced suppression when given in low doses.

30 The present invention also provides kits for enhancing the immunity of a host to a pathogen. These kits may include any one or more vaccines according to the present invention in combination with a composition for delivering the vaccine to a host and/or a device, such as a syringe, for delivering the vaccine to a host.



The vaccine according to the invention can be administered in a conventional active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation, and in such amount as will be prophylactically effective, i.e. the amount of immunizing antigen or  
5 recombinant microorganism capable of expressing the antigen that will induce immunity in humans against challenge by the pathogenic virus or bacteria, such virulent Ebola virus, HIV, hepatitis A, B, C, D, and E virus, and bacillus tuberculous. Immunity is defined as the induction of a significant level of protection after vaccination compared to an unvaccinated human.

10 The vaccine of the present invention, i.e. the recombinant virus, may be administered to a host, preferably a human subject, via any pharmaceutically acceptable routes of administration. The routes of administration include, but are not limited to, intramuscular, intratracheal, subcutaneous, intranasal, intradermal, rectal, intramucosally, oral and parental  
15 route of administration. Routes of administration may be combined, if desired, or adjusted depending upon the type of the pathogenic virus to be immunized against and the desired body site of protection.

The route of administration can be particularly important in influencing the nature of induced immunity, and the degree of protection. For example,  
20 while parenteral administration may only activate a systemic immune response, whereas the oral route provides, in addition, mucosal immune response. The ability of the recombinant viruses of the present invention to elicit a mucosal immunity renders its application important in mucosally and sexually transmitted infection.

25 Doses or effective amounts of the recombinant virus may depend on factors such as the condition, the selected viral or bacterial antigen, the age, weight and health of the host, and may vary among hosts. In general, one skilled in the art understands that the amount of virus particles to be administered depends, for example, on the number of times the vaccine is  
30 administered and the level of response desired.

The appropriate titer of the recombinant virus of the present invention to be administered to an individual is the titer that can modulate an immune response against the viral or bacterial antigen and elicits antibodies against the pathogenic virus or bacteria from which the antigen is derived. An  
35 effective titer can be determined using an assay for determining the activity of

immunoeffector cells following administration of the vaccine to the individual or by monitoring the effectiveness of the therapy using well known *in vivo* diagnostic assays. For example, a prophylactically effective amount or dose of a recombinant adenovirus of the present invention may be in the range of  
5 from about 100  $\mu$ l to about 10 ml of saline solution containing concentrations of from about  $1 \times 10^4$  to  $1 \times 10^8$  plaque forming units (pfu) virus/ml. When other plasmid DNA vectors are used, 1-1000  $\mu$ g per administration is the preferred dose range. The dose may be the same for priming and boosting immunizations or it may be desired to alter quantity of recombinant viruses  
10 provided in the boosting phase as compared to the initial priming dose. The dose of an inoculum of the recombinant virus of the present invention is dictated by and dependent upon the unique characteristics of the particular recombinant virus and the particular immunologic effect to be achieved, as is well-recognized by the skilled artisan.

15

#### **7. Methods of Enhancing the Immunity of a Host to Pathogens**

The present invention also provides methods for enhancing the immunity of a host to pathogens with the recombinant viruses described above.

20

In one embodiment, the method is provided for enhancing the immunity of a host to a pathogenic virus. The method comprises: administering to the host a recombinant virus in an amount effective to induce an immune response. The recombinant virus comprises: an antigen sequence heterologous to the benign virus and encoding a viral antigen from  
25 a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus; and an immuno-stimulator sequence heterologous to the benign virus that encodes an immuno-stimulator whose expression in the host enhances the  
30 immunogenicity of the viral antigen. The recombinant virus may preferably be replication-incompetent and not cause a malignancy naturally associated with the pathogenic virus in the host.

The recombinant virus may be administered to the host via any pharmaceutically acceptable route of administration. The recombinant virus  
35 may be administered to the host via a route of intramuscular, intratracheal,

subcutaneous, intranasal, intradermal, intramucosally, rectal, oral and parental administration.

In another embodiment, a method is provided for immunizing a host against a pathogenic virus with multiple antigens that elicit strong and long-  
5 lasting immune response to the multiple antigens. The method comprises: administering to the host a recombinant virus in an amount effective to induce an immune response. The recombinant virus comprises: a plurality of antigen sequences heterologous to the recombinant virus, each encoding a different  
10 of the antigen sequences eliciting an immune response directed against the viral antigen and cells expressing the viral antigen in the host upon infection of the host by the recombinant virus. The recombinant virus may preferably be replication-incompetent and not cause malignancy that is naturally associated with the pathogenic virus(es) in the host.

15 Optionally, the recombinant virus may also comprise one or more immuno-stimulator sequences heterologous to the recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen.

In yet another embodiment, a method is provided for immunizing a  
20 host against a pathogenic virus by using multiple genetic vaccines or viruses. Multiple recombinant viruses may carry different antigens in each recombinant virus. The multiple recombinant viruses may be administered simultaneously or step-wise to the host.

The method comprises: administering to a host a first and second  
25 recombinant viruses in an amount effective to induce an immune response, wherein antibodies are produced. The first recombinant benign virus comprises: an antigen sequence heterologous to the first recombinant virus that encodes a viral antigen from a pathogenic virus, expression of the viral antigen eliciting an immune response directed against the viral antigen and  
30 cells expressing the viral antigen in the host upon infection of the host by the recombinant virus. The second recombinant virus comprises: an immuno-stimulator sequence heterologous to the second recombinant virus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the viral antigen. The first and second recombinant viruses

may preferably be replication-incompetent and not cause malignancy naturally associated with the pathogenic virus in the host.

According to the embodiment, the first and second recombinant virus may be any of a benign virus, such as replication-incompetent adenovirus, adeno-associated virus, SV40 virus, retrovirus, herpes simplex virus, Alpha virus, Venezuelan Equine Encephalitis (VEE) virus and vaccinia virus. Optionally, both the first and second recombinant viruses may be replication-incompetent adenovirus. Also optionally, one of the first and second recombinant viruses may be recombinant adenovirus and the other may be recombinant vaccinia virus.

In yet another embodiment, a method is provided for enhancing the immunity of a host to a pathogen. The method comprises: administering to the host a recombinant virus and one or more immuno-stimulators. The recombinant virus may be any of the recombinant viruses described above. In particular, the recombinant virus comprises one or more antigen sequences heterologous to the recombinant virus that encode one or more antigens from the pathogen. Expression of the antigen elicits an immune response directed against the antigen and cells expressing the antigen in the host upon infection of the host by the recombinant virus. The recombinant virus is preferably replication-incompetent and does not cause a malignancy naturally associated with the pathogen in the host. The pathogen may be a pathogenic virus such as HIV, hepatitis virus and Ebola virus, a pathogenic bacteria or parasite.

According to this embodiment, the immuno-stimulator may be any molecule that enhances the immunogenicity of the antigen expressed by the cell infected by the recombinant virus. Preferably, the immuno-stimulator is a cytokine, including, but not limited to interleukin-2, interleukin-8, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, and combinations thereof.

The cytokine may be administered into the host in a form of purified protein. Alternatively, the cytokine may be administered in a form of expression vector that expresses the coding sequence of the cytokine upon transfecting or transducing the cells of the host.

According to any of the above embodiments of the methods, the method may further comprise: administering to the host the recombinant virus

again to boost the immune response. Such a booster inoculation with the recombinant virus is preferably conducted several weeks to several months after the primary inoculation. To insure sustained high levels of protection against infection or an efficacious treatment of the disease(s) caused by infection of the pathogen, it may be helpful to re-administer the booster immunization to the host at regular intervals, for example, once every several years. The recombinant virus administered in the booster immunization may be the same as or different from the recombinant virus administered in the primary immunization.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a plasmid vector that encodes the same or different antigen(s) as that (or those) encoded by the recombinant virus. The plasmid vector is preferably a eukaryotic plasmid expression vector that expresses the antigen(s) upon transfection of the cells in the host.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a second recombinant virus to boost the immune response and/or to minimize neutralizing effects of the host's immune system on the recombinant viruses.

Optionally, the second recombinant virus comprises a second antigen sequence from a second pathogen that is different from the first antigen sequence comprised in the first recombinant virus administered in the primary immunization. Preferably, the second antigen sequence encodes the same type of antigen as that encoded by the first antigen sequence but from a different strain, serotype, or subtype/clade of the same pathogen. Alternatively, the second antigen may be a different type of antigen compared to the first antigen, for example, the first antigen being a surface protein and second antigen being a core protein of the same or different pathogen.

Also according to any of the above embodiments of the methods, the method may further comprise: administering to the host a viral vector prior to, concurrently, or post the administration of any of the above embodiment of the recombinant virus to minimize neutralizing effects of the host's immune system on the recombinant virus. Preferably, the viral vector is administered post the administration of the recombinant virus.

The viral vector may be the native progenitor of the recombinant virus. For example, the viral vector may be the wildtype adenovirus type 5 (Ad5) whereas the recombinant virus is a genetically modified Ad5.

5        Optionally, the viral vector may be the wildtype of or a genetically modified virus that is a different serotype of the recombinant virus. For example, the recombinant virus may be a genetically modified Ad5 whereas the viral vector is the wildtype of or a genetically modified adenoviral vector serotype other than Ad5, for example, serotype 1-4 or 6-51. It is noted that other serotypes discovered and/or classified later also fall within the scope of  
10      the invention.

For example, the recombinant virus is a recombinant Ad5 encoding one or more heterologous antigens and/or an immunostimulator while the viral vector may also be a recombinant adenovirus encoding the same or different antigens and/or the immunostimulator but of different serotype (e.g., Ad2,  
15      Ad4, Ad9, Ad12, Ad35 and Ad40). Such a serotype rotation is believed to enhance expression of the transgenes and increase immunogenicity of the vaccines. To verify this belief, a wildtype non-Ad5 vector can be administered to mice first and the levels of anti-adenovirus antibody are measured by ELISA 3 weeks after the injection. The recombinant Ad5 encoding a  
20      heterologous antigen (e.g., HBV core protein) is then administered to the mice 4-5 weeks after the primary injection. The levels of antibody against the heterologous antigen (e.g., the HBV core protein) can be measured 4-5 weeks after the secondary injection.

Also optionally, the viral vector may be a different virus from the  
25      recombinant virus. For example, the recombinant virus may be a genetically modified Ad5 whereas the viral vector is a genetically modified AAV, SV40 virus, retrovirus, herpes simplex virus, Alpha virus, Venezuelan Equine Encephalitis (VEE) virus or vaccinia virus. The viral vector may or may not comprise a heterologous antigen sequence. Preferably, the viral vector may  
30      comprise another antigen sequence which is the same or different from the antigen sequence carried by the recombinant virus.

Also optionally, the viral vector may be a chimeric vector modified based on the native progenitor of the recombinant virus. For example, if the native progenitor of the recombinant virus is adenovirus type 5, the viral vector  
35      may be a chimeric adenovirus type 5 with certain regions of the backbone

changed from type 5 to the corresponding regions from other adenovirus serotypes. This approach is believed to be advantageous because of the ease of cloning when only a portion of the backbone with the corresponding one of another serotype. This may be accomplished by constructing a shuttle vector by including Ad5 fiber DNA and switching the Ad5 fiber DNA partially or completely with that from another serotype of adenovirus. As shown in **Figure 62**, a right shuttle vector pR-Ad.5/35-6m is constructed to replace the fiber region of Ad5 with that of Ad35. This right shuttle vector can be combined with a left shuttle vector and the Ad5 backbone to generate a chimeric Ad5 vector.

Up to date, 51 serotype of human adenovirus have been identified and divided into six subgroups from A to F. The adenovirus entry into the cells is a two- step process consisting of virus attachment to the membrane via the Ad fiber knob, followed by internalization upon binding of the penton base RGD motifs to  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins on the cell surface. De Long et al. (1999) J. Clin. Microbiol. 37:3940; Sallusto et al. (1994) J. Exp. Med. 179:1109; Huang et al. (1995) J. Virol. 69:2257-2263; and Mathias et al. (1994) J. Virol. 68:6811-6814. The adenovirus fiber is considered to be a crucial mediator for high efficiency binding to target cells. Subgroup C, Ad5 fiber uses the coxsackievirus and adenovirus receptor (CAR) to mediate the high affinity binding. Nemerow (1999) Mol. Biol. Rev. 63:725-734. In CAR-deficient cells, Ad5 attachment occurs at much lower efficiency through alternative pathways involving interactions between the fiber and the MHC class I heavy chain  $\alpha 2$  domain or between the penton and cellular integrins. Bergelson et al. (1997) Science 275:1320-1323.

The adenovirus fiber can be divided into three domains. The conserved N-terminal tail contains the sequences responsible for association with the penton base. De Long et al. (1999) J. Clin. Microbiol. 37:3940. The rod-like fiber shafts contains number of repeats ranging from 6 to 23 form the  $\beta$  sheets. Davison et al. (1999) J. Virol. 73:4513. The C-terminal contains globular knob domains, which both fiber shaft and knob are involved in the primary receptor interaction. Huang et al. (1996) J. Virol. 70:4502.

According to the present invention, for example, the fiber knob (i.e., the head), shaft, and/or penton base (i.e., the tail) in the backbone of adenovirus type 5 can be replaced by the corresponding region(s) of the

backbone from adenovirus serotype 1-4, and 6-51. **Figure 63** shows various embodiments of the chimeric vectors having the individual domains of the Ad5 fiber regions substituted with the corresponding domains of Ad35 fiber region.

Preferably, the knob domain of the fiber region of Ad5 is swapped with the  
5 corresponding one from another serotype of adenovirus since the knob domain is believed to determine the receptor-ligand interaction.

For example, the recombinant virus is a recombinant Ad5 encoding one or more heterologous antigens and/or an immunostimulator while the chimeric viral vector may also be a recombinant Ad5 encoding the same or  
10 different antigens and/or the immunostimulator but having a fiber region from adenovirus of different serotype (e.g., Ad2, Ad4, Ad9, Ad12, Ad35 and Ad40). Such a serotype rotation is believed to enhance expression of the transgenes and increase immunogenicity of the vaccines. To verify this belief, an Ad5 vector carrying GFP can be administered to mice first and the levels of anti-  
15 GFP antibody are measured by ELISA 4 weeks after the injection. Another recombinant Ad5 also carrying GFP but having a fiber region from different serotype adenovirus (e.g., Ad9, Ad 11, or Ad35) is then administered to the mice 4-5 weeks after the primary injection. The levels of antibody against GFP can be measured 4-5 weeks after the secondary injection.

20 The methods described above may be used for prevention or treatment of diseases. In the method of treatment, the administration of the recombinant viruses of the present invention may be for either "prophylactic" or "therapeutic" purpose. When provided prophylactically, the recombinant virus is provided in advance of any symptom. The prophylactic administration  
25 of the recombinant virus serves to prevent or ameliorate any subsequent infection or disease. When provided therapeutically, the recombinant virus is provided at (or after) the onset of a symptom of infection or disease. Thus, the present invention may be provided either prior to the anticipated exposure to a disease-causing agent or after the initiation and/or progression of the  
30 infection or disease.

It is noted that the innovative approaches of the present invention may also be employed in the construction of cancer vaccines. For example, sequences encoding tumor-specific antigens may substitute the antigen sequence encoding viral antigen in any of the above embodiments of the  
35 recombinant virus and methods of using the same. Expression of tumor-



specific antigens in the host should elicit specific immune response for prevention in patients with an increased risk of cancer development (i.e., preventive immunization) or to enhance the treatment of cancer with other therapeutics, prevention of disease recurrence after primary surgery (anti-  
5 metastatic vaccination), or as a tool to expand the number of CTL in vivo, thus improving their effectiveness in eradication of diffuse tumors (treatment of established disease). In addition, the methods of the present invention may elicit an immune response in a patient that is enhanced ex vivo prior to being transferred back to the tumor bearer (i.e., the adoptive immunotherapy).

10 Also according to any of the above embodiments of the methods, the method may further comprise: harvesting serum from the host after the administration of the recombinant virus. The harvested serum should contain antibodies against the antigen(s) encoded by the recombinant virus. Optionally, the method may further comprise: isolating antibody or antibodies  
15 against the pathogen from the host after the administration of the recombinant virus. The harvested serum or isolated antibody can be stored for certain periods of time for further uses. For example, a healthy human volunteer can serve as the host and the serum or antibody collected from him/her may be administered back to him/herself or a different person later to in anticipation or  
20 in the event of infection of the pathogen as prophylactic or therapeutic agent by eliciting passive immunity against the pathogen. Optionally, the host may be a non-human animal and the serum harvested or antibody isolated from the animal immunized by the recombinant virus may be used as a prophylactic or therapeutic agent to treat a human or non-human animal in anticipation or  
25 in the event of infection of the pathogen such as in the outbreak of biological warfare.

It should be noted that modifications and changes can be made in the DNA sequence of any of the above-described antigens and immuno-  
stimulators included in the recombinant virus and still maintain functional  
30 equivalence of the mutant. For example, wildtype codons for the above-described antigens can be replaced with codons that are preferred by the host to be immunized, e.g., a human. Synthetic polynucleotide can be made to include the preferred codons for the "humanized" antigens. Such a humanization process may be advantageous in that by using the preferred  
35 codons, translation efficiency of the antigens expressed by the recombinant

virus can be significantly improved, which in turn can result in higher levels of humoral and/or cellular immune responses in the host. All of the above-described mutants fall within the scope of the present invention.

Standard procedures for endonuclease digestion, ligation and  
5 electrophoresis are carried out in accordance with the manufacturer's or  
supplier's instructions. Standard techniques are not described in detail and  
will be well understood by persons skilled in the art. Practicing the present  
invention employs, unless otherwise indicated, conventional methods of  
virology, microbiology, molecular biology and recombinant DNA techniques  
10 within the skill of the art. Such techniques are explained fully in the literature.  
See e.g. Sambrook, et al. *Molecular Cloning: A laboratory Manual*; *DNA  
Cloning: A Practical Approach*, vol I & II (D. Glover ed.); *Oligonucleotide  
Synthesis* (N. Giat, ed.); *Nucleic Acid Hybridization* (B. Hames & S. Higgins,  
eds., Current Edition); *Transcription and Translation* (B. Hames & S. Higgins,  
15 eds., Current Edition); *Fundamental Virology*, 2nd Edition, vol. I & II (B. N.  
Fields and D. M. Knipe, eds.)

The following examples are provided to illustrate the present invention  
without, however limiting the same thereto.

## EXAMPLES

The following procedures are described to illustrate how to make a genetic vaccine of the present invention against various pathogenic viruses.

5 The genetic vaccine is based on an adenoviral vector with modified antigens derived from the pathogenic virus (e.g., Ebola virus, Hepatitis B virus and HIV) inserted into the adenoviral backbone. Additionally, the recombinant adenovirus also carries multiple genes encoding various cytokines. The recombinant adenovirus is replication-incompetent but still retains adenoviral infectivity.

10 It is noted that genetic vaccine against other pathogenic viruses, bacteria and parasites may be constructed by one with ordinary skill in the art following similar procedures described in details below.

15

### 1. Genetic Vaccine against Ebola Virus

Embodiments of the genetic vaccine against Ebola virus and methods of their construction are described in detail as follows.

20 1) Genetic modification of the Ebola virus membrane glycoproteins

The modifications are carried out using standard molecular genetic manipulation techniques, such as restriction enzyme digests and polymerase chain reaction (PCR).

25 The glycoproteins of Ebola virus are modified to produce the optimal antigen for Ebola virus vaccine. Two modified forms of the GP proteins are constructed to have inactivated immunosuppressive and infectious mechanisms, but retain full natural antigenicity of the wild-type glycoproteins. The mRNA editing signal is deleted to prevent the production of the secreted glycoprotein (sGP), which is immunosuppressive; and (2) the proteolytic cleavage site of the glycoprotein precursor is deleted to prevent the formation of the functional glycoproteins (GP1 and GP2). Sanchez, A., et al., *Proc Natl Acad Sci U.S.A.* 93(8):3602-7 (1996). In one form the immunosuppressive peptide region is deleted to prevent its function, and in the other form, the immunosuppressive peptide motif is split in order to destroy its function, but

35

retain its immunogenicity. These steps produce effective and safe antigens for the vaccine.

The envelope glycoproteins (GP) of the Ebola virus are synthesized as a single precursor protein and cleaved into the two subunits (GP1 and GP2) by a cellular enzyme (furin) during transport. Volchkov, V.E., et al., *Proc Natl Acad Sci U.S.A.*, 95(10):5762-7 (1998). This proteolytic cleavage is essential for the formation of the mature glycoproteins and the release of the fusion peptide located at the C-Terminus of the cleavage site. The mature glycoproteins are incorporated into virions as trimers (each monomer is a heterodimer of GP1 and GP2 linked by a disulfide bond). Sanchez, A., et al., *J. Virol* 72(8):6442-7 (1998). The glycoproteins of Ebola virus are the major proteins exposed on the viral membrane surface, and are responsible for initiating virus entry into host cells. Therefore, they are a primary target for neutralizing antibodies.

The glycoprotein cleavage site is composed of five basic amino acid residues (RRTRR [SEQ ID NO: 10]) at position 501 from the start site of the open reading frame. The Ebola virus glycoprotein cleavage site is similar to the conserved sequences found in glycoproteins of other viruses, such as in the envelope protein of RSV or MuLV. We have previously shown that deletions or point mutations at these basic amino acid residues can block cleavage and render the glycoproteins non-functional in RSV. Dong, J.Y., et al., *J. Virol* 66(2):865-74 (1992).

To destroy the infective functions of the Ebola virus glycoprotein, the five basic amino acid residues in the cleavage site are deleted. This deletion is introduced into the Ebola virus GP cDNA using PCR amplification. Alternatively, the cleavage site can be altered, such as by site specific mutation resulting in elimination of cleavage.

Another important feature of the Ebola virus is that two forms of glycoproteins are synthesized from a single gene, a secreted form (sGP) and a membrane-bound form (GP). The two forms are generated as a result of an alternative RNA editing event at a sequence of seven uridines (at location 1020-1028 from the start site), which is highly conserved among all four Ebola virus subtypes. Sanchez, A., et al., *Proc Natl Acad Sci U.S.A.* 93(8):3602-7 (1996). The sGP is synthesized from un-edited mRNA and likely has immunosuppressive functions. The GP is synthesized from an edited mRNA

and likely has immunosuppressive functions. The GP is synthesized from an edited mRNA with insertion in one of the seven uridines. This RNA editing causes a frame-shift and results in a translation of the second reading frame that encodes the complete transmembrane glycoprotein (GP2).

5 To prevent the synthesis of sGP, the RNA editing site is modified from UUUUUUU [SEQ ID NO: 2] to UUCUUCUU [SEQ ID NO: 3]. In the cDNA, the equivalent sequence is AAAAAAA [SEQ ID NO: 4] and AAGAAGAA [SEQ ID NO: 5], respectively. This modification accomplishes two things: (1) all mRNAs encode only the GP (equivalent to the edited form with -1 frame shift);  
10 and (2) UUUUUUU [SEQ ID NO: 6] encodes the same amino acid residues as UUCUUC [SEQ ID NO: 7], but prevents the possibility of further polymerase slipping at the stretch of the six uridines. The additional editing would cause deletion of one more uridine and further (-2) frame shifting. The mechanism of this modification is diagrammed in **Figure 2**.

15 A third modification may be introduced into the Ebola virus glycoprotein relating to a deletion of the immunosuppressive (IS) peptide located in GP2. The IS peptide motif (amino acid 585-609, from the start site) is highly conserved in filoviruses and has a high degree of homology with a motif in the glycoproteins of oncogenic retroviruses that has been shown to be  
20 immunosuppressive. Volchkov, V.E., et al., *FEBS Lett* 305(3):181-4 (1992); Will, C., et al., *J. Virol* 67(3):1203-10 (1993); Mitani, M., et al., *Proc Natl Acad Sci U.S.A.* 84(1):237-40 (1987); Gatot, J.S., et al., *J. Biol Chem* 273(21):12870-80 (1998); Denner, J., et al., *J Acquir Immune Defic Syndr Hum Retrovirol* 12(5):442-50 (1996). First, a ten amino acid deletion is  
25 introduced in the core region of the motif (between amino acid 590-600) to remove its function. Second, each half of the motif is reversed and duplicated to destroy function and increase antigenicity. It is believed that antibodies against the IS peptide may inhibit the immunosuppressive function of the Ebola viruses during an infection. The basic strategy of this modification is  
30 diagrammed in **Figures 3A-3C**.

As illustrated in **Figures 3A-3C**, modification of the immunosuppressive peptide (IS) is made on the GP2 gene. **Figure 3A** illustrates the wild type GP. **Figure 3B** illustrates GP with the 10 amino acid deletion of the IS peptide. **Figure 3C** illustrates the IS peptide, which is split,  
35 reversed and duplicated.

With these modifications, Ebola virus glycoproteins are generated that are non-functional, not immunosuppressive, yet they retain the natural antigenicity of GP. These modified GP sequences are used to generate antigens in the vaccines of the present invention against Ebola virus.

5 DNA sequences of the resulting altered GP genes are confirmed by sequence analysis. The modified GP sequences are then cloned into plasmid vectors containing DNA elements necessary for efficient expression of these GPs in hostian cells. Expression and correct localization to the cellular membrane is determined by transient transfections of HeLa or 293 cells and  
10 analyzed by Western blot and FACS, using polyclonal antibodies from hyperimmunized equine serum and anti-horse secondary antibodies labeled with horse radish peroxidase (HRP) or fluorescent tags, respectively.

2) Construction of a series of replication-defective adenoviral vaccines  
15 that mediate high levels of expression of the modified Ebola virus GPs

The vaccines of the present invention utilize a recombinant benign virus to carry modified antigens of Ebola virus to trick the host into mounting a robust immune defense against the Ebola virus. The preferred benign virus is  
20 a replication-defective adenovirus. These vectors are an excellent choice for vaccine expression, for several reasons. First, adenoviral vectors direct high levels of antigen expression that provides strong stimulation of the immune system. Second, the antigen that they express is processed and displayed in the transduced cells in a way that mimics pathogen-infected cells. This phase  
25 is believed to be very important in inducing cellular immunity against infected cells, and is completely lacking when conventional vaccination approaches are used. Third, adenoviral vectors infect dendritic cells which are very potent antigen-presenting cells. Diao, J. et al., *Gene Ther* 6(5):845-53 (1999); Zhong, L., et al., *Eur J Immunol* 29(3):964-72 (1999); Wan, Y., et al., *Int J*  
30 *Oncol* 14(4):771-6 (1999); Wan, Y., et al., *Hum Gene Ther* 8(11):1355-63 (1997). Fourth, these vectors can be engineered to carry immunoenhancing cytokine genes to further boost immunity. Fifth, adenoviruses naturally infect airway and gut epithelial cells in humans, and therefore the vaccine may be delivered through nasal spray or oral ingestion. And finally, the adenoviral  
35 vectors of this invention are safe because they are replication-defective

and have been used in high doses ( $10^9$  to  $10^{12}$  i.p./dose) in clinical trials for gene therapy studies. Gahery-Segard, H., et al., *J. Clin Invest* 100(9):2218-26 (1997); Bellon, G., et al., *Hum Gene Ther* 8(1):15-25 (1997); Boucher, R.C., et al., *Hum Gene Ther* 5(5):615-39 (1994). Indeed, even live viruses have been safely used in military recruits to prevent common colds.

This vector-construction system is also used to establish complex vectors that express multiple genes or regulatory mechanisms. For example, the vector construct is used to express multiple cytokines along with Ebola GP antigens in a single complex vector to further enhance the immune induction. Alternatively, antigens and cytokines are placed in separate vectors. This enables the manipulation of different combinations of cytokines and antigens by co-transduction (infection) with two or three vectors.

Construction of the adenoviral vectors is diagramed in **Figure 4**. The cDNA encoding a modified GP(s) is cloned into the left-end (E1 region) of the adenovirus genome using a shuttle vector pLAd (**Figure 4A** left side), resulting in a shuttle vector pLAd/EBO-GP. The pLAd/EBO-GP vector contains the left end of the adenoviral genome including the left long terminal repeats L-TR and the adenoviral packaging signal  $\psi$ . Genes encoding cytokines such as IL-2 and IL-4 are inserted into E4 region of the adenovirus vector using the shuttle vector pRAd (**Figure 4A**, right side), resulting in a shuttle vector pRAdIL2,4. The pRAdIL2,4 contains the right end of the adenoviral genome including the right long terminal repeats R-TR.

To construct an adenoviral vector carrying the GP gene only, the shuttle vector pLAd/EBO-GP is digested with appropriate restriction enzymes such as Xba I. The fragment containing the GP gene is ligated to an adenoviral backbone and pRAd vector.

To construct an adenoviral vector carrying both the GP gene in the E1 region and cytokine genes in the E4 region, both pLAd/EBO-GP and pRAdIL2,4 are linearized and ligated to the backbone of the adenovirus (**Figure 4B**).

To generate recombinant adenoviral vectors, the ligated vector genome is transfected into 293 cells, in which only the correctly ligated genome with the two adenoviral terminal repeats can replicate and generate infectious viral particles. Human 293 cells (Graham et al., *J. Gen. Virol.*, 36: 59-72 (1977)), available from the ATCC under Accession No.: CRL1573, has adenovirus E1a and E1b genes stably integrated in its genome. The 293 cells

supplement the essential E1 gene of adenovirus that has been deleted from the vector backbone. The final vector has E1, E3 and partial E4 deleted and can only replicate in 293 cells, but not in target cells. The adenoviral vectors are amplified in 293 cells and purified by ultracentrifugation in cesium chloride  
5 gradients. Titers of vectors are determined by serial dilutions and counting of the infectious particle (ip) after infection of 293 cells.

3) Determination of immune responses to the genetic vaccine

10 An *in vitro* assay is used to quantitate the amount of neutralizing antibodies developed in response to the vaccine. The assay is based on a retroviral vector system which is based on a Moloney Murine Leukemia virus system. Vectors and packaging cells expressing GAG and POL proteins have been extensively characterized and are commercially available. A packaging  
15 vector construct that carries a  $\beta$ -galactosidase gene as a reporter is used. A novel vector construct expressing the membrane form of the Ebola virus GP is co-transfected with the  $\beta$ -Gal reporter vector resulting in a GAG-POL packaging cell line, which generates retroviral vector particles with the Ebola virus GP instead of its original envelope protein.

20

4) Determination of which modified GP antigen provides better production of neutralizing antibodies in animal models

The adenoviral vaccine vectors carrying the two GP variants are tested  
25 for their ability to induce an immune response to the Ebola virus GP in CD-1 mice (Charles River Laboratories; outbred stock of Swiss mice from Rockefeller Institute). Specifically, the neutralizing antibody titers and cytolytic T-lymphocyte (CTL) activities to the Ebola virus GP antigens induced by the GP variants with and without the IS motif are compared. Three groups of 30  
30 8-week old mice are injected subcutaneously with  $10^5$  ip of adenoviral vectors expressing GP variant 1 (with IS peptide deleted), GP variant 2 (with IS peptide split and inverted) and  $\beta$ -Galactosidase (control vector), respectively. Six mice from each group are sacrificed (by CO<sub>2</sub> asphyxiation and cervical dislocation) at 1, 2, 4, 8 and 16 weeks post-vaccination, and their blood and



spleens are harvested. In addition, 6 mice are mock-vaccinated with saline and sacrificed 2 days later to provide preimmunization controls.

From mice injected with the control  $\beta$ -Gal vector, tissue sections from the sites of the vector injection are taken, fixed, and stained with the X-gal solution to determine the number and type of vector-transduced cells at various time-points post-infection. In addition, hemolysin staining is performed to determine the degree of infiltration of various immune cells (neutrophils, macrophages, monocytes, etc.) at the site of the vector delivery.

Sera from vaccinated animals is assayed for total GP-binding antibodies using a standard 96-well plate ELISA protocol, as has been described. Van Ginkel, F.W., et al., *Hum Gene Ther* 6(7):895-903 (1995); Van Ginkel, F.W., et al., *J Immunol* 159(2):685-93 (1997). Neutralizing activity of the sera is analyzed by monitoring the infectious activity of the Ebola virus GP-pseudotyped retroviral vector (Wool-Lewis, et al., *J. Virol*, 72(4):3155-60 (1998)) on HeLa cells after the vector has been incubated with various serum concentrations. Expression of  $\beta$ -galactosidase in infected cell lysates serves as an indicator of the neutralizing activity of the serum (the lower the  $\beta$ -gal activity, the more EBO- $\beta$ -Gal vectors have been neutralized) and is measured using a very sensitive fluorogenic substrate (Galacto-Light kit J) and a fluorescence plate reader. Anti-GP serum-neutralized infection rates are compared to infection rates in the absence of serum and in the presence of non-GP activated serum.

Cytotoxic lymphocytes (CTLs) are extracted from mouse spleen as previously described. Van Ginkel, F.W., et al., *Hum Gene Ther* 1995; 6(7):895-903; Dong, J.Y., et al., *Hum Gene Ther* 1996;7(3):319-31. They are mixed with a constant number of detached LnCaP cells (prostate carcinoma cells of epithelial origin) transduced with an adenoviral vector carrying an unmodified Ebola virus GP protein. Ratios of effector: target cells of 10:1, 3:1, and 1:1 are used. The cells are seeded into 96-well plates, and 24 hour later all unattached cells (which include all of the effector CTLs and dead or dying LnCaP cells) are removed, and the remaining viable (adherent) cells are quantitated by the MTT (3-(4,5-dimethylthiazol-20-yl) 2,5-diphenyl tetrazolium bromide) cleavage assay. This assay has been employed in detecting the lymphocyte cytotoxic activity (Ni, J., et al., *J Clin Lab Anal* 1996;10(1):42-52)

and compares favorably with the radioactive assays in terms of sensitivity, reliability and speed.

5) Immuno-enhancing functions of multiple cytokines  
 5 and their effects on the efficacy of the genetic vaccines

To augment the effects of the vaccine, a vector-mediated gene transfer to express the immunoenhancing cytokines, such as IL2, IL4, IL12, INF- $\gamma$ , and GM-CSF is used. Initially, each cytokine is separately cloned or the  
 10 cytokines are cloned in various combinations into adenoviral vectors separate from the vectors encoding viral antigens. The immunoenhancing effects of individual cytokines or their combinations are studied by co-infecting with a vector encoding the cytokine and the vector carrying the antigens. The titers of serum antibodies are compared, as well as the time it takes to reach  
 15 effective titers in animals inoculated with vaccines in combination with different cytokine-expressing vectors. These experiments allow the determination of whether immunoenhancing cytokines induce higher levels of antibodies, shorten the induction time, and prolong the immunity against the Ebola virus.

20 After determining the best-performing modified GP variant, the extent that the immune response elicited by it is enhanced by co-delivery to the immunization site of vectors carrying various cytokines is analyzed. Interleukin-2, either by itself or in combination with IL-4 or IL-12, has been demonstrated to strongly enhance the activation and proliferation of cytotoxic  
 25 T-cells, natural killer (NK) cells and B-cells. Michael, B.N., et al., *Cell Immunol* 1994;158(1):105-15; Bruserud, O., et al., *Eur J. Haematol* 1992;48(4):221-7; Jacobsen, S.E., et al., *Res Immunol* 1995;146(7-8):506-14; Wolf, S.F., et al., *Res Immunol* 1995;146(7-8):486-9; Tepper, R.I., *Res Immunol* 1993;144(8):633-7; O'Garra A., et al., *Res Immunol* 1993;144(8):620-5;  
 30 Ohe, Y., et al., *Int J Cancer* 1993;53(3):432-7; Delespesse, G., et al., *Res Immunol* 1995;146(7-8):461-6. [36-43]. INF- $\gamma$  stimulates the humoral immune response and increases the permeability of the blood vessel walls at the site of its secretion (Chensue, S.W., et al., *J Immunol* 154(11):5969-76 (1995); Szente, B.E., et al., *Biochem Biophys Res Commun* 203(3):1645-54 (1994);  
 35 Adams, R.B., et al., *J. Immunol* 150(6):2356-63(1993)), while Gm-CSF

activates and attracts macrophages and other professional APCs to the site of the infection. Bober, L.A., et al., *Immunopharmacology* 29(2):111-9 (1995); Dale, D.C., et al., *Am J. Hematol* 57(1):7-15 (1998); Zhao, Y., et al., *Chung Hua I Hsueh Tsa Chih* 77(10): 32-6 (1997).

5                Six groups of 30 8-week old mice are injected subcutaneously with a mixture of  $5 \times 10^4$  ip of the selected GP variant vector and  $5 \times 10^4$  ip of one of the following vectors: Ad- $\beta$ -Gal, Ad-IL2, Ad-IL2/IL4, Ad-IL2/IL12, Ad-IFN- $\gamma$  and Ad-GM-CSF.

10                Six mice from each group are sacrificed and analyzed at 1, 2, 4, 8 and 16 weeks as described in Example 4. Analysis of total IgG is performed using ELISA, neutralizing activity is assayed as interference with the ability of EBO-GP pseudotyped retroviral vector to infect HeLa cells, and anti-GP CTL activity is performed by mixing spleen-extracted CTLs with target LnCaP cells transduced with Ad-EBO-GP construct as described in Example 4. Levels of various cytokines in the serum are also quantitated by ELISA using available commercial assays. In some cases, these assays can distinguish between human and murine versions of the same cytokine, providing direct information on the expression levels of cytokines delivered using Adenovirus vectors and how they correlate with the development of the immune response.

20                After the individual cytokines are analyzed, those that performed best are tested in combinations. Four groups of 30 8-week old mice are injected subcutaneously with a mixture of  $5 \times 10^4$  i.p. of the selected GP variant vector and  $5 \times 10^4$  i.p. of up to 3 selected cytokine-expressing vectors (if fewer than 3 cytokine vectors are used, i.p. counts are made up with Ad- $\beta$ -Gal vector). Six mice from each group are sacrificed at weeks 1, 2, 4, 8 and 16, and analyzed as described above.

30                To verify the robust and reproducible nature of the immune response to the GP vector and multiple cytokines in different species, the experiment as described above is reproduced in rabbits. Five groups of six white New Zealand rabbits are injected into the thigh muscle with one of the following vector combinations: the Ad- $\beta$ -Gal vector ( $10^6$  ip), the selected GP vector ( $2.5 \times 10^5$  ip plus  $7.5 \times 10^5$  ip of Ad- $\beta$ -Gal), and three cytokine vector combinations ( $2.5 \times 10^5$  of each cytokine vector) plus the GP vector ( $2.5 \times 10^5$  ip), as described above. The animals are bled 2 days prior to vaccination (pre-immune bleed) and then according to the schedule described above. 5 to 10 ml of blood will

be extracted per session. Analysis is performed in a similar fashion to that of mice (see above).

Because genes coding for human cytokines are used in mouse and rabbit models, it is possible that their immune systems will have a non-  
5 identical (to human) response to those proteins. However, a high degree of homology exists between human and mouse cytokines and their receptors, and published reports on experiments using human or other hostian cytokines in mice indicate a high level of equivalency. If necessary, species-specific  
10 versions of these cytokines can be obtained and cloned into the adenoviral vectors of the present invention for species-targeted cytokine activity studies.

6) Optimizing the efficiency and rates of administration of the vaccine and conducting safety and pathogen challenge studies in non-primate and primate animal models

15

After determining the best combinations of the cytokines and antigens, the final version of the vaccine vectors are constructed. These complex recombinant adenoviral vectors deliver combinations of cytokines and antigens into target cells using a single vector. Dose-titer analysis in mice and  
20 rabbits are conducted to identify the lowest dose required to generate maximal levels of immune responses. Different routes of vaccine administration, such as intramuscular and intravenous injection, oral ingestion and nasal sprays are compared. For safety studies, dose escalation experiments in mice and rabbits are conducted until toxicity is observed or  
25 until levels ten times the effective dose have been reached. Finally, additional safety and pathogen challenge experiments are conducted in primates.

**2. Genetic Vaccine Against HIV**

Specific embodiments of the genetic vaccines against HIV and  
30 methods of their construction are described in detail as follows.

A. Construction of replication-defective adenoviral vaccines against HIV

1) *Ad-E.T.R/IL2*

An adenoviral vector, Ad-E.T.R/IL2, was constructed to carry coding sequences for multiple HIV antigens including Env, Tat, and Rev proteins, and interleukin-2 (IL-2) in the same vector. Expression of the HIV antigens and IL-2 is separately controlled by promoters located in different regions of the adenoviral vector. This design is believed to be able to ensure high level expression of both the viral antigens and the immuno-stimulator IL-2 and to enhance immunogenicity of the adenoviral vaccine. As shown by experimental data presented in the next section, this adenoviral vector is capable of eliciting strong humoral immune response in animals against HIV antigens.

The adenoviral vector, Ad-E.T.R/IL2, was constructed using strategies similar to those for constructing the adenoviral vaccines against Ebola virus as described in detail above. Briefly, EcoRI/XhoI restriction fragment from HIV-1 strain BH10 (HIV-1 or HTLV-IIIB, clade B, Accession No: M15654), which encodes wildtype envelope gp160 (full length gp 120 and gp41), full length wildtype Tat and full length wild type Rev, was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E.T.R (**Figure 16A**). DNA sequence of this EcoRI/XhoI restriction fragment [SEQ ID NO: 14] is shown in **Figure 38**.

The sequence encoding IL-2 (with a silent mutation CTA to CTT at amino acid position 79 to delete the XbaI site) was inserted into E4 region of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pRAd-ORF6-IL2 (**Figure 16B**). DNA sequence encoding this mutated IL-2 (IL-2ΔX) [SEQ ID NO: 15] is shown in **Figure 39**.

Both pLAd-E.T.R and pRAd-ORF6-IL2 were linearized using appropriate restriction enzymes such as Xba I and EcoRI and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E.T.R/IL2.

## 30 2) *Ad-3C/E<sup>m</sup>ΔCAT<sup>300</sup>-G*

Another adenoviral vector, Ad-3C/E<sup>m</sup>ΔCAT<sup>300</sup>-G, was constructed to carry coding sequences for multiple HIV antigens including a modified Env (gp160) with deletion of the cleavage site between gp120 and gp41 and the cytosolic domain and Gag proteins, and three different cytokines (IL-2 with silent mutation CTA to CTT at amino acid position 79 to delete the XbaI site,

INF- $\gamma$ , and GMCSF) in the same vector. Expression of the HIV antigens and the cytokines is separately controlled by promoters located in different regions of the adenoviral vector. This design is believed to be able to ensure high level expression of both the viral antigens and the immuno-stimulators and to enhance immunogenicity of the adenoviral vaccine. As shown by experimental data presented in the next section, this adenoviral vector is capable of eliciting strong humoral immune response in animals against HIV antigens.

The adenoviral vector, Ad-3C/E<sup>m</sup> $\Delta$ C $\Delta$ T<sup>300</sup>-G, was constructed using strategies similar to those for constructing the adenoviral vaccines against Ebola virus as described in detail above. Briefly, the sequence from HIV-1 strain BH10 that encodes Env/gp160 (nucleotide position 5580-7850) was modified to delete the sequences encoding the cleavage site (REKR [SEQ ID NO: 11] encoded by nucleotide at position 7101-7112) and the cytosolic domain of 100 amino acids in length (encoded by nucleotide at position 7850-8150), and then, along with the sequence encoding a full length Gag, inserted into the right end (E4 region) of the adenoviral genome using a shuttle vector. DNA sequence of this modified Env (E<sup>m</sup> $\Delta$ C $\Delta$ T (BH10) [SEQ ID NO: 16] and that of the full length Gag [SEQ ID NO: 17] (amino acid sequence of which is SEQ ID NO: 18, **Figure 41B**) are shown in **Figures 40** and **41A**, respectively.

These two HIV antigens are expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub>, and SA<sub>2</sub>. To facilitate efficient cloning of various gene fragments, a cloning vector SD/SA1.2.3 was constructed to include a retroviral SD site and multiple retroviral SA sites, SA<sub>1</sub>, SA<sub>2</sub>, SA<sub>3</sub> and SA<sub>4</sub>. In this example, the SD and SA sites were derived from Moloney murine leukemia virus (MMLV) and their sequences are shown below:

SD site (MMLV nt 204-210): AGGTAAG [SEQ ID NO: 72]; and

SA<sub>1-4</sub> site (MMLV nt 560-568): CTGCTGCAG [SEQ ID NO: 73].

Each of the SD site, and the SA<sub>1</sub>, SA<sub>2</sub>, SA<sub>3</sub> and SA<sub>4</sub> (SA<sub>1-4</sub>) sites which share the same sequence was inserted into the multiple cloning site of a cloning vector pSP73 by using standard PCR mutagenesis. As illustrated in **Figure 37**, SA<sub>1</sub> was inserted immediately downstream from SD site, followed by SA<sub>2</sub>, SA<sub>3</sub> and SA<sub>4</sub>. To test the levels of expression of multiple genes via the SD/SA mechanism, the GFP (green fluorescence protein) gene was inserted between

SD/SA<sub>1</sub> and SA<sub>2</sub>, SA<sub>2</sub> and SA<sub>3</sub>, SA<sub>3</sub> and SA<sub>4</sub>, and after SA<sub>4</sub>. The ratio of expression levels in these four sites is 10:1:5:4.

DNA sequences encoding E<sup>m</sup>ΔCΔT and Gag were inserted into the cloning vector SD/SA1.2.3 after SD/SA<sub>1</sub>, and SA<sub>2</sub>, respectively. The resulting vector was digested with EcoRV and XhoI and the fragment containing E<sup>m</sup>ΔCΔT and Gag was inserted into an adenoviral shuttle vector, resulting in pRAd-ORF6-cmv- E<sup>m</sup>ΔCΔT<sup>300</sup>-G (**Figure 17A**). Shuttle vectors capable of expressing other proteins (as shown below) via the retroviral SD/SA mechanism were constructed using the same strategy.

Sequences encoding multiple immuno-stimulators, including IL-2 (with a silent mutation caused by deletion of Xba I site), INF-γ, and GMCSF, were inserted into E1 region of the adenoviral genome using a shuttle vector. These three immuno-stimulators are expressed separately from another CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at three splicing acceptor sites, SA<sub>1</sub>, SA<sub>2</sub>, and SA<sub>3</sub>. The shuttle vector produced is designated pLAd-3C (**Figure 17B**).

Both pRAd-ORF6-cmv- E<sup>m</sup>ΔCΔT<sup>300</sup>-G and pLAd-3C were linearized using appropriate restriction enzymes such as Xba I and EcoRI and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-3C/E<sup>m</sup>ΔCΔT<sup>300</sup>-G.

### 3) *Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R-G*

Yet another adenoviral vector, Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R-G, was constructed to carry coding sequences for multiple HIV antigens from strain pNL4-3 (Accession No: M19921) including a modified Env (gp160 with deletion of the cleavage site and the cytoplasmic domain of 33 amino acids in length), full length Rev and Gag proteins, and three different cytokines (IL-2 with silent mutation CTA to CTT at amino acid position 79 to delete the XbaI site, INF-γ, and GMCSF) in the same vector. Expression of the HIV antigens and the cytokines is separately controlled by promoters located in different regions of the adenoviral vector. This design is believed to be able to ensure high level expression of both the viral antigens and the immuno-stimulators and to enhance immunogenicity of the adenoviral vaccine. As shown by experimental data presented in the next section, this adenoviral vector is

capable of eliciting strong humoral immune response in animals against HIV antigens.

The adenoviral vector, Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R-G, was constructed using strategies similar to those for constructing the adenoviral vaccines against Ebola virus as described in detail above. Briefly, the sequence from HIV-1 strain pNL4-3 that encodes Env/gp160 (nucleotide position 6221-8686) was modified to delete the sequences encoding the cleavage site (encoded by nucleotide at position 7736-7747) and the cytosolic domain (encoded by nucleotide at position 8687-8785) in length, and then, along with sequences encoding full length Tat, Rev, and Gag (from HIV strain BH10), inserted into the right end (E4 region) of the adenoviral genome using a shuttle vector.

These three HIV antigens are expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at three splicing acceptor sites, SA, SA<sub>2</sub>, and SA<sub>3</sub>. The shuttle vector produced is designated pRAd-E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R-G (**Figure 18**). DNA sequence encoding the modified Env, and full length Tat and Rev [SEQ ID NO: 19] is shown in **Figure 42**. DNA and amino acid sequences of the full length Gag from HIV strain BH10 [SEQ ID NO: 17] are shown in **Figures 41A and 41B**, respectively.

The shuttle vectors, pRAd-E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R.-G and pLAd-3C (**Figure 17B**) were linearized using appropriate restriction enzymes such as Xba I and EcoRI and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-3C/E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R.-G.

#### 25 4) *Ad-E<sup>m</sup>ΔV<sub>1,2</sub> ΔC ΔT<sup>99</sup>.T.R-IL2/G.IL2*

Yet another adenoviral vector, Ad-E<sup>m</sup>ΔV<sub>1,2</sub> ΔC ΔT<sup>99</sup>.T.R/G.IL2, was constructed to carry coding sequences for multiple HIV antigens from HIV-1 strain pNL4-3. The sequence from HIV-1 strain pNL4-3 that encodes Env/gp160 (nucleotide position 6221-8686) was modified to delete the sequences encoding the V1 and V2 loops at position 6602-6796 nt and insert nucleotide sequence GGA GCT GGT [SEQ ID NO: 12] that encodes amino acid sequence GAG [SEQ ID NO: 13]. This HIV Env/gp160 was also modified to delete the cleavage site encoded by nucleotide at position 7736-7747 (ΔC) and the 33-aa cytosolic domain encoded by nucleotide at position 8687-8785



( $\Delta T^{99}$ ). Along with the sequences encoding full length Rev (R) and Tat (T), the modified *env* was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector. DNA sequence encoding the insert ( $E^m\Delta V_{1,2}\Delta C\Delta T.T.R$ ) [SEQ ID NO: 20] is shown in **Figure 43**.

5            Additionally, IL-2 (with a silent mutation caused by deletion of Xba I site, DNA SEQ ID NO: 15) was inserted downstream from the modified *env*. Both the modified Env and IL-2 are expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>/SA<sub>3</sub>. The shuttle vector produced is  
10            designated pLAd-  $E^m\Delta V_{1,2}\Delta C\Delta T.T.R$ -IL2 (**Figure 19A**).

             Sequences encoding IL-2 (with a silent mutation caused by deletion of Xba I site, DNA SEQ ID NO: 15) and Gag from HIV-1 strain BH10 (nt 112-1650, DNA SEQ ID NO: 17) were inserted into E4 region of the adenoviral genome using a shuttle vector. These two proteins are expressed separately  
15            from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>. The shuttle vector produced is designated pRAd-ORF6-G.IL2 (**Figure 19B**).

             Both pLAd-cmv-  $E^m\Delta V_{1,2}\Delta C\Delta T.T.R$ -G and pRAd-ORF6-G.IL2 were linearized using appropriate restriction enzymes and ligated to the backbone  
20            of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad- $E^m\Delta V_{1,2}\Delta C\Delta T.T.R$ -G/G.IL2.

#### 5)        *Ad- $E^m\Delta C.T.R.N$ /G.IL2*

             Yet another adenoviral vector, Ad- $E^m\Delta C.T.R.N$ /G.IL2, was constructed  
25            to carry coding sequences for multiple HIV antigens from HIV-1 strain BH10. The sequence from HIV-1 strain BH10 that encodes full length Env/gp160 (nucleotide position 5580-8150), Tat, Rev, and Nef was modified by deleting the sequence encoding the cleavage site of Env and inserting a SpeI restriction site. DNA sequence of this insert [SEQ ID NO: 21] is shown in  
30            **Figure 44**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd- $E^m\Delta C.T.R.N$  (**Figure 20**).

             Both pLAd- $E^m\Delta C.T.R.N$  and pRAd-ORF6-G.IL2 (**Figure 19B**) were linearized using appropriate restriction enzymes and ligated to the backbone

of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔC.T.R.N/G.IL2.

6) *Ad-E<sup>m</sup>ΔC.N/G.IL2*

5 Yet another adenoviral vector, Ad-E<sup>m</sup>ΔC.N/G.IL2, was constructed to carry coding sequences for multiple HIV antigens from HIV-1 strain BH10. The sequence from HIV-1 strain BH10 that encodes full length Env/gp160 (nucleotide position 5580-8150, with preceding Kozak sequence), Tat, Rev, and Nef was modified by deleting the sequences encoding the cleavage site  
10 of Env, Tat and Rev, and inserting a SpeI restriction site. DNA sequence of this insert [SEQ ID NO: 22] is shown in **Figure 45**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>ΔC.N (**Figure 21**).

Both pLAd-E<sup>m</sup>ΔC.N and pRAd-ORF6-G.IL2 (**Figure 19B**) were  
15 linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔC.N/G.IL2.

7) *Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.T/G.IL2*

20 Yet another adenoviral vector, Ad-E<sup>m</sup>ΔC ΔT<sup>300</sup>.T/G.IL2, was constructed to carry coding sequences for multiple HIV antigens from HIV-1 strain BH10. The sequence from HIV-1 strain BH10 that encodes full length Env/gp160 (nucleotide position 5580-8150) was modified by deleting the sequence encoding the cleavage site and a 300 nt sequence encoding the  
25 cytosolic domain, but still including sequence for full length Tat (T). DNA sequence of this insert [SEQ ID NO: 23] is shown in **Figure 46**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>ΔC ΔT<sup>300</sup>.T (**Figure 22**).

Both pLAd-E<sup>m</sup>ΔC ΔT<sup>300</sup>.T and pRAd-ORF6-G.IL2 (**Figure 19B**) were  
30 linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔC ΔT<sup>300</sup>.T/G.IL2.

8) *Ad-E<sup>m</sup>ΔC/E<sup>m</sup>ΔC*

Yet another adenoviral vector, Ad-E<sup>m</sup>ΔC/ E<sup>m</sup>ΔC, was constructed to carry coding sequences for two copies of a modified Env from HIV-1 strain BH10. The sequence from HIV-1 strain BH10 that encodes full length Env/gp160 (nucleotide position 5580-8150, preceding Kozak sequence) was modified by deleting the sequence encoding the cleavage site. DNA  
 5 sequence of the modified Env [SEQ ID NO: 24] is shown in **Figure 47**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>ΔC (**Figure 23A**).

The DNA sequence encoding the modified Env (E<sup>m</sup>ΔC) [SEQ ID NO:  
 10 24] was also inserted into E4 region of the adenoviral genome using a shuttle vector, resulting in shuttle vector pRAd-ORF6-E<sup>m</sup>ΔC (**Figure 23B**).

Both pLAd-E<sup>m</sup>ΔC and pRAd-ORF6-E<sup>m</sup>ΔC were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔC/ E<sup>m</sup>ΔC.

15

#### 9) *Ad-E<sup>m</sup>.V3<sup>m</sup>/G.IL-2*

Yet another adenoviral vector, Ad-E<sup>m</sup>.V3<sup>m</sup>/G.IL-2, was constructed to carry coding sequences for modified HIV-1 Env having multi-clade V3 loops and Gag, and IL-2. Sequences encoding V3 loop from clade B, A, C, D, E, F,  
 20 and G within Group M of HIV-1 are shown in **Figure 48**. As shown in **Figure 48**, for clade B (HIV-1 strain BH10) DNA sequence encoding V3 loop, nt 885-992 [SEQ ID NO: 25], was chosen. In this particular embodiment, for clade A (HIV-1 strain 192UG037WHO.01083hED) DNA sequence encoding V3 loop, nt 888-992 [SEQ ID NO: 26], was chosen. For clade C (HIV-1 strain  
 25 192BR025WHO.01093hED) DNA sequence encoding V3 loop, nt 876-980 [SEQ ID NO: 27], was chosen. For clade D (HIV-1 strain 192UG024.2) DNA sequence encoding V3 loop, nt 888-989 [SEQ ID NO: 28], was chosen. For clade E (HIV-1 strain 193TH976.17) DNA sequence encoding V3 loop, nt 894-998 [SEQ ID NO: 29], was chosen. For clade F (HIV-1 strain 193BR020.17)  
 30 DNA sequence encoding V3 loop, nt 888-992 [SEQ ID NO: 30], was chosen. For clade G (HIV-1 strain 192RU131.9) DNA sequence encoding V3 loop, nt 885-989 [SEQ ID NO: 31], was chosen.

The DNA sequences encoding V3 loops from HIV clade A, C, D, E, F, and G were ligated by PCR to form a single fragment containing multiclade V3

loops. Primers for cloning these V3 loops from their cognate HIV clades are listed in **Figure 57**. Since V3 loop of HIV clade B is already contained in the backbone of HIV-1 gp120, the cloned V3 loops from clade A, C, D, E, F, and G were inserted after V3 loop of clade B.

5           **Figure 24** illustrate a process for generating the ligated multiclade V3 loops by PCR and subsequent cloning into a construct encoding a modified gp120 of clade B. As illustrated in **Figure 24**, each of the gene fragments encoding the envelope V3 loop region from clade A, C, D, E, F, and G was individually amplified by PCR using a set of forward and reverse primers listed in  
10   **Figure 57**. Parameters for the PCR cycles are the following:

denature:     94 °C for 1 min;  
annealing:    50 to 60 °C for 30 sec; and  
extension:     72 °C for 1 min;  
for 20 cycles.

15           The PCR product encoding V3 loop of one clade was ligated with another using PCR. For example, the PCR products encoding V3 loops of clade A and C were mixed together, ligated and amplified by PCR using the primers 1 and 4 as shown in **Figure 24**, producing an A/C fragment. Similarly, a PCR product encoding the ligated V3 loops of clade D and E was generated  
20   using primers 5 and 8, producing a D/E fragment; and clade F and G using primers 9 and 12 (**Figure 24**), producing a F/G fragment.

              Still referring to **Figure 24**, the A/C and D/E fragments were ligated by PCR using primers 1 and 8 and cloned into a vector at EcoRI and BamHI sites. The F/G fragment was restriction digested with BamHI and XbaI and  
25   fused with the sequence A/C/D/E to generate the multi-clade sequence ACDEFG (V3<sup>m</sup>).

              To generate two repeats of the multi-clade ACDEFG sequence, the final PCR product encoding the multi-clade ACDEFG sequence was restriction digested with Aval (at primer 1 and 12) and re-ligated head-to-tail, yielding the  
30   two repeat multiclade sequence 2x V3<sup>m</sup>. The DNA sequence encoding V3<sup>m</sup> or 2x V3<sup>m</sup> was then inserted after the sequence encoding V3 loop of clade B in a construct encoding gp120 which was modified as follows.

              DNA sequence encoding Env (nt 5580-8150) from HIV strain BH10 (clade B) was modified by a) deleting the sequence encoding the cleavage  
35   site (nt 7101-7112); b) deleting V1 and V2 loops (nt 5961-6161) and inserting

nucleotide sequence GGA GCT GGT [SEQ ID NO: 12] that encodes amino acid sequence GAG [SEQ ID NO: 13]; c) inserting the multi-clade V3 loop (V3<sup>m</sup>) sequence at position nt 6572; and d) replacing gp41 transmembrane domain sequence with a GPI anchor sequence encoding glycosylphosphatidylinositol, SWLLLLLLSLSLLQATDFMSL [SEQ ID NO: 9]. DNA sequence encoding this modified Env [SEQ ID NO: 32] (the amino acid sequence of which is SEQ ID NO: 33, **Figure 49B**) is shown in **Figure 49A**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>.V3<sup>m</sup> (**Figure 25**).

Both pLAd-E<sup>m</sup>.V3<sup>m</sup> and pRAd-ORF6-G.II.2 (**Figure 19B**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>.V3<sup>m</sup>/G.II.2.

10) *Shuttle Vector pLAd-E<sup>m</sup>.2xV3<sup>m</sup>*

To increase the expression level of the multi-clade V3 loops, the sequence encoding two repeats of V3<sup>m</sup> sequence (2xV3<sup>m</sup>, constructed above) was inserted into the sequence encoding the modified Env described in section 9) above. The resulting shuttle vector is designated pLAd-E<sup>m</sup>.2xV3<sup>m</sup> and is shown in **Figure 26**.

11) *Shuttle Vectors encoding p17 and/or p24*

In nature the Pr55 Gag protein can be processed into four different proteins, p17MA, p24CA, p7NC, and p6. The p17MA protein remains associated with the inner side of the lipid envelope, and plays an important role in anchoring of envelope to the viral particle. The p24CA protein of all retroviruses contains a major homology region (MHR) that is required for efficient viral replication and particle production. Elispot data obtained implicates that p17MA (or p17) and p24CA (or p24) may have contributed significantly the specific CTL response in the Pr55 gag protein in peptide mapping experiments. According to the present invention, these HIV structural proteins are expressed by the recombinant virus to elicit specific CTL response to HIV infection. Further, these structure proteins can be modified to include a signal peptide (e.g., the HIV gp120 signal peptide encoded by SEQ ID NO: 74:

atgagagtgaaggagaaatatcagcactgtggagatgggggtggagatggggcaccatgctccttggga  
 tgttgatgatctgtagtgt) sequence which facilitates the secretion of these  
 intracellular proteins by the infected cells. Moreover, by adding a membrane  
 anchoring domain (e.g, the HIV gp41 transmembrane domain encoded by  
 5 SEQ ID NO: 75:

ttattcataatgatagtaggaggcttggtagggttaagaatagttttgctgtactttctgtagtgaatagagtagg  
 cagggatattcaccattatcgtttcagaccacccatcccgaggga) to the secreted form  
 of the HIV protein, such a modified HIV structural protein is rendered  
 membrane bound, which better presents the HIV antigen to the body's  
 10 immune system. These modifications may confer a much stronger  
 immunogenicity to the mutant antigen than the native antigen which are  
 trapped intracellularly.

Adenoviral shuttle vectors were constructed to encode the processed  
 Gag proteins, p17, p24, and p17/24, each in three different forms: natural  
 15 form, secreted form and membrane bound form.

DNA sequences of p17/p24 in the three forms [SEQ ID NOs: 34-36]  
 are shown in **Figure 50A** (corresponding amino acid sequences [SEQ ID NOs:  
 37-39], **Figure 50B**) and were each inserted into E4 region of the adenoviral  
 genome using a shuttle vector, resulting in shuttle vector pRad-ORF6-p17/24  
 20 (natural form, **Figure 27A**), pRad-ORF6-p17/24sec (secreted form, **Figure**  
**27B**), and pRad-ORF6-p17/24MB (membrane-bound form, **Figure 27C**),  
 respectively.

DNA sequences of p17 in the three forms [SEQ ID NOs: 40-42] are  
 shown in **Figure 51A** (corresponding amino acid sequences [SEQ ID NOs: 43-  
 25 45], **Figure 51B**) and were each inserted into E4 region of the adenoviral  
 genome using a shuttle vector, resulting in shuttle vector pRad-ORF6-p17  
 (natural form, **Figure 28A**), pRad-ORF6-p17sec (secreted form, **Figure 28B**),  
 and pRad-ORF6-p17MB (membrane-bound form, **Figure 28C**), respectively.

DNA sequences of p24 in the three forms [SEQ ID NOs: 46-48] are  
 30 shown in **Figure 52A** (corresponding amino acid sequences [SEQ ID NOs: 49-  
 51], **Figure 52B**) and were each inserted into E4 region of the adenoviral  
 genome using a shuttle vector, resulting in shuttle vector pRad-ORF6-p24  
 (natural form, **Figure 29A**), pRad-ORF6-p24sec (secreted form, **Figure 29B**),  
 and pRad-ORF6-p24MB (membrane-bound form, **Figure 29C**), respectively.

The pLAd- and pRAd-shuttle vectors constructed above can be combined in a combinatorial way to generate a wide variety of recombinant adenoviral vectors. The following are just a few examples of such recombinant adenoviral vectors.

5

12) *Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17/24MB*

**Figures 30A-B** illustrate the construction of a recombinant adenoviral vector encoding modified Env containing two copies of multi-clade V3 loops and p17/p24 in membrane-bound form. As illustrated in **Figures 30A-B**, pLAd-E<sup>m</sup>.2xV3<sup>m</sup> (details of the vector shown in **Figure 26**) and pRAd-ORF6-p17/24MB (details of the vector shown in **Figure 27C**) were linearized using EcoR1 and Xba1 restriction enzymes and ligated to the backbone of the adenovirus, resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17/24MB.

15

13) *Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17MB*

**Figures 31A-B** illustrate the construction of a recombinant adenoviral vector encoding modified Env containing two copies of multi-clade V3 loops and p17 in membrane-bound form. As illustrated in **Figures 31A-B**, pLAd-E<sup>m</sup>.2xV3<sup>m</sup> (details of the vector shown in **Figure 26**) and pRAd-ORF6-p17MB (details of the vector shown in **Figure 28C**) were linearized using EcoR1 and Xba1 restriction enzymes and ligated to the backbone of the adenovirus, resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p17MB.

25

14) *Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p24MB*

**Figures 32A-B** illustrate the construction of a recombinant adenoviral vector encoding modified Env containing two copies of multi-clade V3 loops and p24 in membrane-bound form. As illustrated in **Figures 32A-B**, pLAd-E<sup>m</sup>.2xV3<sup>m</sup> (details of the vector shown in **Figure 26**) and pRAd-ORF6-p24MB (details of the vector shown in **Figure 29C**) were linearized using EcoR1 and Xba1 restriction enzymes and ligated to the backbone of the adenovirus, resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>.2xV3<sup>m</sup>/p24MB.

30

15) *Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T./p17/24sec*

DNA sequence encoding Env (including Tat1 (nt 5189-5403) and Tat2 (7734-7779)) from HIV strain BH10 was modified by a) deleting the sequence encoding the cleavage site (nt 7101-7112); b) deleting V1 and V2 loops (nt 5961-6161) and inserting nucleotide sequence GGA GCT GGT [SEQ ID NO: 12] that encodes amino acid sequence GAG [SEQ ID NO: 13]; c) inserting two copies of the multi-clade V3 loop (2xV3<sup>m</sup>) sequence at position nt 6572; and d) deleting the cytosolic domain of 100 amino acids in length (encoded by nucleotide at position 7850-8150). DNA sequence encoding this modified Env [SEQ ID NO: 52] (the amino acid sequence of which is SEQ ID NO: 53, **Figure 53B**) is shown in **Figure 53A**, and was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T (**Figure 33**).

Both pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T and pRAd-ORF6-p17/24sec (**Figure 27B**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T./p17/24sec.

16) *Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T./p17/24MB*

Both pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T (**Figure 33**) and pRAd-ORF6-p17/24MB (**Figure 27C**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T./p17/24MB.

17) *Ad-E<sup>m</sup>ΔCΔT<sup>99</sup>.2xV3<sup>m</sup>.T.R/p17/24sec*

DNA sequence encoding Env (including Tat1 (nt 5189-5403), Rev1 (nt 5328-5403), Tat2 (7734-7779) and Rev2 (7734-8008)) from HIV strain BH10 was modified by a) deleting the sequence encoding the cleavage site (nt 7101-7112); b) deleting V1 and V2 loops (nt 5961-6161) and inserting nucleotide sequence GGA GCT GGT [SEQ ID NO: 12] that encodes amino acid sequence GAG [SEQ ID NO: 13]; c) inserting two copies of the multi-clade V3 loop (2xV3<sup>m</sup>) sequence at position nt 6572; and d) deleting the cytosolic domain of 33 amino acids in length (nt 8687-8785). DNA sequence encoding this modified Env [SEQ ID NO: 54] (the amino acid sequence of which is SEQ ID NO: 55, **Figure 54B**) is shown in **Figure 54A**, and was



inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector, resulting in a shuttle vector pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T.R (**Figure 34**).

Both pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T.R and pRAd-ORF6-p17/24sec (**Figure 27B**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>99</sup>.2xV3<sup>m</sup>.T.R/p17/24sec.

18) *Ad-E<sup>m</sup>ΔCΔT<sup>99</sup>.2xV3<sup>m</sup>.T.R/p17/24MB*

Both pLAd-E<sup>m</sup>ΔCΔT<sup>99</sup>.2xV3<sup>m</sup>.T.R (**Figure 34**) and pRAd-ORF6-p17/24MB (**Figure 27C**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>99</sup>.2xV3<sup>m</sup>.T.R/p17/24MB.

19) *Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T/G.PI*

Peptide mapping and Elispot data indicate that specific regions of the Gag protein may play significant roles in eliciting CTL response in animals immunized with the adenoviral vectors of the present invention. To facilitate efficient expression of p17MA and p24CA by the adenoviral vector, DNA sequence encoding the protease (PI, DNA SEQ ID NO: 56, **Figure 55A**; amino acid SEQ ID NO: 57, **Figure 55B**) from the *pol* region of HIV strain BH10 was inserted into a region downstream from the sequence encoding Gag in a shuttle vector pRAd-ORF6-G.PI (**Figure 35**). As illustrated in **Figure 35**, Gag and PI are expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>/SA<sub>3</sub>.

Both pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T (**Figure 33**) and pRAd-ORF6/G.PI (**Figure 35**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T/G.PI.

20) *Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T/G-PI*

Alternatively, the HIV protease PI was expressed as a fusion protein with Gag by inserting a C residue at position nt1410 to allow *pol* to be read within the same reading frame of *gag*. DNA [SEQ ID NO: 58] and amino acid

[SEQ ID NO: 59] sequences of the Gag-PI fusion protein are shown in **Figures 56A and 56B**, respectively. As illustrated in **Figure 36**, Gag and PI are expressed from the same CMV promoter within the same reading frame. The resulting shuttle vector is designated as pRAd-ORF6/G-PI.

5           Both pLAd-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T (**Figure 33**) and pRAd-ORF6/G-PI (**Figure 36**) were linearized using appropriate restriction enzymes and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector Ad-E<sup>m</sup>ΔCΔT<sup>300</sup>.2xV3<sup>m</sup>.T/G-PI.

10       21) *pRAd-ORF6-Gag/PI-RT*

          DNA sequence encoding the protease (PI) and reverse transcriptase (RT) from the *pol* region of HIV strain BH10 was inserted into a region downstream from the sequence encoding Gag in a shuttle vector pRAd-ORF6-Gag/PI-RT (**Figure 58**). As illustrated in **Figure 58**, Gag and PI-RT are  
15       expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>/SA<sub>3</sub>.

          The right shuttle vector pRAd-ORF6-Gag/PI-RT can be combined with any of the left shuttle vector (pLAd) described above to generate a  
20       recombinant adenoviral vector.

          22) *pRAd-ORF6-Gag-PI-RT*

          Alternatively, PI-RT was expressed as a fusion protein with Gag by inserting a C residue at position nt1410 to allow *pol* to be read within the same  
25       reading frame of *gag*. As illustrated in **Figure 59**, Gag and PI-RT are expressed from the same CMV promoter within the same reading frame. The resulting shuttle vector is designated as pRAd-ORF6-Gag-PI-RT.

          The right shuttle vector pRAd-ORF6-Gag-PI-RT can be combined with any of the left shuttle vector (pLAd) described above to generate a  
30       recombinant adenoviral vector.

          23) *pRAd-ORF6-Gag/Pol*

          DNA sequence encoding the HIV enzymes PI, RT and IN from the *pol* region of HIV strain BH10 was inserted into a region downstream from the

sequence encoding Gag in a shuttle vector pRAd-ORF6-Gag/Pol (**Figure 60**).

As illustrated in **Figure 60**, Gag and Pol are expressed separately from a CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>/SA<sub>3</sub>.

5           The right shuttle vector pRAd-ORF6-Gag/Pol can be combined with any of the left shuttle vector (pLAd) described above to generate a recombinant adenoviral vector.

24) *pRAd-ORF6-Gag-Pol*

10           Alternatively, Pol was expressed as a fusion protein with Gag by inserting a C residue at position nt1410 to allow *pol* to be read within the same reading frame of *gag*. As illustrated in **Figure 61**, Gag and Pol are expressed from the same CMV promoter within the same reading frame. The resulting shuttle vector is designated as pRAd-ORF6-Gag-Pol.

15           The right shuttle vector pRAd-ORF6-Gag-Pol can be combined with any of the left shuttle vector (pLAd) described above to generate a recombinant adenoviral vector.

20           B. Immune responses of animals to the adenoviral vaccine against HIV antigens

Experimental mice were inoculated with the adoviral vaccine constructed above, Ad.tat.env.IL2 (also designated as "Ad-E.T.R/IL2" as described above, section A, subsection 1)), to elicit immune response to the HIV antigens expressed by this vector. Immunogenicity of the adenoviral vector was determined by measuring titers of antibody against HIV tat and env.

**Figures 6 and 7** show the immunogenicity of Ad.tat.env.IL2 against the HIV Env protein in two groups of mice, respectively. These groups of C57BL/6 mice (supplied by Charles River Laboratories, Wilmington, MA) were injected intramuscularly with 10<sup>7</sup> pfu Ad.tat.env.IL2 on different dates as indicated in the figures. Blood (about 150-500 µl for each animal) was collected from four animals every two weeks following inoculation and serum was prepared. At 77 days post-inoculation, these mice were re-challenged with an additional 10<sup>7</sup> pfu of Ad.tat.env.IL2. Blood was collected from three animals every day following secondary challenge. Titers of antibody elicited

against HIV tat and env were determined by ELISA against Ad.tat.env.IL2-infected HeLa cell lysates.

Briefly, lysates of the HeLa cells infected with Ad.tat.env.IL2 were prepared as follows. HeLa cells were infected with Ad.tat.env.IL2 at a multiplicity of infection (MOI) of 20. Forty-eight hours post infection, HeLa cells were harvested and resuspended in a buffer that contained 1% TritonX-100. A post-nuclear supernatant was obtained by centrifuging the lysates at 15,000 x g for 5 min. The lysates were diluted to 10 µg/ml for coating wells of ELISA plates. Standard ELISA assays were performed to measure OD450 of the sera and relative titers of antibody against HIV tat and env proteins were calculated by normalizing against the mean of the OD450 of mouse pre-immunization sera.

As shown in **Figure 6**, the three mice in this group had strong immune responses to the HIV antigens expressed by the adenoviral vector Ad.tat.env.IL2, with the highest titer of antibody against HIV antigens reached in about 42 days post inoculation. The second inoculation with Ad.tat.env.IL2 boosted the immune response again and very high titers were achieved within about 5 days of the second inoculation.

As shown in **Figure 7**, the three mice in this group also had strong immune responses to the HIV antigens expressed by the adenoviral vector Ad.tat.env.IL2, with the highest titer of antibody against HIV antigens reached in about 70 days post inoculation. The second inoculation with Ad.tat.env.IL2 boosted the immune response again and very high titers were achieved within about 5 days of the second inoculation.

**Figures 12 A-B** show the antibody production elicited by the recombinant adenoviral vectors Ad.3C.env.gag (also designated as "Ad-3C/E<sup>m</sup>ΔCΔT<sup>300</sup>-G" as described above, section A, subsection 2)) in mice. C57BL/6 mice were injected intramuscularly with 10<sup>7</sup> pfu Ad.3C.env.gag. At 77 days post-inoculation, these mice were re-challenged with an additional 10<sup>7</sup> pfu Ad.3C.env.gag. Relative antibody titers of these mice were determined by ELISA against purified recombinant Gag (obtained from the NIH AIDS Research and Reference Reagent Program; Bethesda, MD) at week 10 post-immunization (or prime) (**Figure 12A**) and week 14 post-prime/week 3 post-boost (**Figure 12B**). As shown in **Figures 12A** and **12B**, the mice inoculated with Ad.3C.env.gag had strong immune responses to the HIV antigen Gag.

**Figures 13 A-B** show the antibody production elicited by the recombinant adenoviral vectors Ad.3C.env.rev.gag (also designated as “Ad-3C/E<sup>m</sup>ΔCAT<sup>89</sup>.T.R-G” as described above in section A, subsection 3)) in mice. C57BL/6 mice were injected intramuscularly with 10<sup>7</sup> pfu Ad.3C.env.rev.gag. At 77 days post-inoculation, these mice were re-challenged with an additional 10<sup>7</sup> pfu Ad.3C.env.rev.gag. Relative antibody titers of these mice were determined by ELISA against recombinant purified Gag at week 10 post-immunization (or prime) (**Figure 13A**) and week 14 post-prime/week 3 post-boost (**Figure 13B**). As shown in **Figures 13A** and **13B**, the mice inoculated with Ad.3C.env.rev.gag had strong immune responses to the HIV antigen Gag.

C. Activation of cytotoxic T lymphocytes (CTL) by immunization with the adenoviral vaccines against HIV antigens

Activation of cytotoxic T lymphocytes (CTL) by immunization with the adenoviral vaccine against HIV antigens was measured by using two independent assays: an IFN $\gamma$  assay and a granzyme A assay. The IFN $\gamma$  and granzyme assays were designed to detect antigen-specific activation of T-cells. IFN $\gamma$  is secreted by activated CTL and T<sub>H</sub>1 helper T cells which function specifically in the cellular immune pathway. Granzyme A is also secreted by activated CTL. The basic approach is to incubate splenocytes with target cells that express antigens of interest and look for secretion of IFN $\gamma$  or granzyme A into the medium.

1) *IFN $\gamma$  assay*

This assay is a modification of the standard <sup>51</sup>Cr-release lytic assay (Current Protocols in Immunology, Coligan et al., eds.) except that the target cells are not radiolabeled prior to incubation with the splenocytes. Detailed procedures for this assay are described in Di Fabio et al. (1994) “Quantitation of human influenza virus-specific cytotoxic T lymphocytes: correlation of cytotoxicity and increased numbers of IFN-gamma-(or IFN $\gamma$ -) producing CD8+ T cells” Int. Immunol. 6:11-9. Briefly, about 1x10<sup>5</sup> splenocytes were incubated with 10<sup>5</sup> target cells (e.g., infected with appropriate viruses carrying the target

antigens) in a total volume of 100  $\mu$ l. Cells were incubated for 4h at 37°C. IFN $\gamma$  was measured by ELISA from 25  $\mu$ l medium.

Activation of CTL in mice inoculated with the adenoviral vaccine against HIV antigens was determined by using the IFN $\gamma$  assay described  
5 above. Briefly, twelve C57BL/6 mice were injected intramuscularly with  $10^7$  pfu Ad.tat.env.IL2. Spleens were harvested from 4 inoculated mice at the time points indicated in **Figures 8A-C**. Splenocytes were activated by incubation with B16-F1 cells (a melanoma cell line from C57BL/6, ATCC No: CRL-6323) that had been infected with Ad.tat.env.IL2. At day seven after  
10 stimulation, activated splenocytes were mixed with B16-F1 cells infected with the indicated viruses. IFN $\gamma$  secretion into the medium was determined by ELISA (R&D Systems, Minneapolis, MN).

**Figures 8A-C** show percent increases in the amount of IFN $\gamma$  secreted into the medium over the period of time ranging from 4-8 weeks post  
15 inoculation. As shown in **Figure 8A**, secretion of IFN $\gamma$  increased significantly in splenocytes of the four mice harvested 4 weeks post inoculation with Ad.tat.env.IL2. In contrast, little increase in IFN $\gamma$  secretion occurred when the splenocytes were incubated with B16-F1 cells infected with an adenoviral vector expressing non-specific protein  $\beta$ -Gal (Ad.lacZ) or uninfected B16-F1  
20 cells.

Secretion of IFN $\gamma$  increased more in the splenocytes of mice harvested 6 weeks post inoculation as shown in **Figure 8B**. Noticeably, there was near 100% increase in secretion of IFN $\gamma$  in splenocytes of mouse 5  
(**Figure 8B**).

Secretion of IFN $\gamma$  increased more dramatically in the splenocytes of mice harvested 8 weeks post inoculation as shown in **Figure 8C**. There was more than 300% increase in secretion of IFN $\gamma$  in splenocytes of mouse 11  
(**Figure 8C**).

These results demonstrate that strong humoral immune responses  
30 against HIV, such as induction of high titer antibody and activation of CTL specifically targeting HIV antigens, have been achieved by inoculating animals with the adenoviral vaccine expressing both HIV viral antigens and an immuno-stimulator such as IL-2. The immune responses resemble those during a recovering of viral infection diseases. These results tend to show

that the genetic vaccines of the present invention that mimics natural viral infection hold great promises as efficacious vaccines for humans against HIV.

2) *Granzyme A assay*

5           Granzyme A assay was performed using a protocol modified from the one described in Deitz et al. (2000) "MHC I-dependent antigen presentation is inhibited by poliovirus protein 3A" *Proc. Natl. Acad. Sci.* 97:13790–13795. The granzyme A assay described in Deitz et al. was a modification of a protocol described in: Kane et al. (1989) "Cytolytic T-lymphocyte response to isolated class I H-2 proteins and influenza peptides" *Nature (London)* 10   340:157–159.

          Granzyme A Assays were performed following similar procedures as for IFN $\gamma$  assays with the following exceptions. Granzyme A secretion into the medium was determined by an enzymatic assay. Units of granzyme A were 15   determined by calculating the slope of activity during the linear phase of the reaction. One unit of granzyme A was defined as the amount of enzyme required to convert the substrate to 1 OD<sub>405</sub> in one hour.

          Briefly, about  $1 \times 10^5$  activated splenocytes and about  $1 \times 10^5$  target cells were incubated together as for the IFN $\gamma$  assays. Granzyme A activity was 20   determined by combining 20  $\mu$ l medium with 180  $\mu$ l reaction mixture (0.2 mM BLT (N- $\alpha$ -benzyloxycarbonyl-L-lysine thiobenzyl ester, Sigma, St. Louis, MO), 0.22 mM DTNB (5,5'-dithio-bis(2-nitrobenzoic acid, Sigma, St. Louis, MO)) in 96-well plates and incubating at room temperature. Absorbance at 405 nm was monitored over a period of several hours. Slopes of enzyme activity were 25   determined for the linear phase of the reaction and converted to units of enzyme.

**Figure 9** shows increases in the amount of granzyme A secreted into the medium for splenocytes of mice harvested 8 weeks post inoculation. As shown in **Figure 9**, secretion of granzyme A increased significantly in 30   splenocytes of the four mice harvested 8 weeks post inoculation with Ad.tat.env.IL2. In contrast, much less granzyme A secretion occurred when the splenocytes were incubated with B16-F1 cells infected with an adenoviral vector expressing non-specific protein  $\beta$ -Gal (Ad.lacZ), an adenoviral vector expressing both hepatitis B surface antigen and IL-2 (Ad.HBsAg/IL2) or

uninfected B16-F1 cells. Similarly, there is little spontaneous granzyme A secretion in these splenocytes not incubated with the target cells.

**Figure 14A** shows the results of the granzyme A assays for series 1 mice at various time points indicated, including week 4, 6, 8 post-immunization and week 12/1, 13/2, 14/3 (prime/boost) post-secondary inoculation with Ad.3C.env.gag.

**Figure 14B** shows the results of the granzyme A assays for series 2 mice at various time points indicated, including week 2, 4, 6, 8 post-immunization with Ad.3C.env.gag.

These results, obtained by using the granzyme A assay independent from the IFN $\gamma$  assay, again demonstrate that strong activation of CTL specifically targeting HIV antigens was induced by inoculating mice with the adenoviral vaccine expressing both HIV viral antigens and an immuno-stimulator such as IL-2. These results also support the belief that the genetic vaccines provided by the present invention hold great promises as efficacious vaccines for humans against HIV.

### 3) *ELISPOT* Assay

ELISPOT assays were performed to determine CTL activation in mice inoculated with the recombinant adenoviral vectors, Ad.3C.env.gag and Ad.3C.env.gag.rev. C57BL/6 mice were inoculated with  $10^7$  pfu Ad.3C.env.gag or Ad.3C.env.gag.rev. Mice were sacrificed at two-week intervals and splenocytes were prepared (see Current Protocols in Immunology, Coligan et al. eds.). At week 11, mice were inoculated with a second dose of  $10^7$  pfu of Ad.3C.env.gag or Ad.3C.env.gag.rev.  $2 \times 10^5$  splenocytes were incubated with  $4 \times 10^4$  MC57G cells (ATCC #CRL-2295) that had been infected with vaccinia viruses expressing either Env, Gag, or Rev, in 96-well, mouse IFN $\gamma$ , ELISPOT plates (R&D Systems, Minneapolis, MN) for 30h. Non-specific activation was monitored following the addition of 4  $\mu$ g/ml PHA (Sigma, St. Louis, MO) instead of antigen-expressing cells. IFN $\gamma$  spots were visualized as per the kit instructions and counted. Wild type and recombinant vaccinia viruses were obtained from the NIH AIDS Research and Reference Reagent Program, Bethesda, MD.

**Figures 15A** shows the ELISPOT results for the four mice in serie1 at week 13/2 post-prime/boost with Ad.3C.env.gag. **Figures 15B** shows the



ELISPOT results for the four mice in serie1 at week 13/2 post-prime/boost with Ad.3C.env.rev.gag. These results indicate that immunization of mice with the genetic vaccines of the present invention induced strong activation of CTL against HIV Gag.

5

### 3. Genetic Vaccine Against Hepatitis B Virus

Embodiments of the genetic vaccine against hepatitis B virus and methods of their construction are described in detail as follows.

#### 10 1) Construction of replication-defective adenoviral vaccines against hepatitis B virus

Two adenoviral vectors, Ad.HBsAg.IL2 and Ad.HBcAg.IL2, were constructed to carry the coding sequences for a hepatitis B surface antigen (HBsAg) and a HBV core antigen (HBcAg), respectively. In the same vector, DNA sequence encoding interleukin-2 (IL-2) was also included and expressed by a promoter different from that for expressing the viral antigen. This design is believed to be able to ensure high level expression of both the viral antigens and the immuno-stimulator IL-2 and to enhance immunogenicity of the adenoviral vaccine. As shown by experimental data presented in the next section, both of these two adenoviral vectors are capable of eliciting strong and long-lasting immune responses in animals against hepatitis B antigens.

15

20

These two adenoviral vectors, Ad.HBsAg.IL2 and Ad.HBcAg.IL2, were constructed using strategies similar to those for constructing the adenoviral vaccines against Ebola virus as described in detail above.

25

#### a) *Ad.HBsAg.IL2*

Briefly, full length HBsAg (with a silent mutation caused by deletion of Xba I site) was inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector pLAd (Figure 4A, left side), resulting in a shuttle vector pLAd-CMV-HBsAg.

30

The sequence encoding IL-2 (with a silent mutation caused by deletion of Xba I site) was inserted into E4 region of the adenoviral genome using the shuttle vector pRAd (Figure 4A, right side), resulting in a shuttle vector pRAd-CMV-IL2.

35

Both pLAd-CMV-HBsAg and pRAd-CMV-IL2 were linearized using appropriate restriction enzymes such as Xba I and EcoRI and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector designated Ad.HBsAg.IL2.

5

a) *Ad.HBcAg.IL2*

Briefly, sequences encoding full length HBsAg (with a silent mutation caused by deletion of Xba I site) and full length HBcAg were inserted into the left end (E1 region) of the adenoviral genome using a shuttle vector pLAd (**Figure 4A**, left side). HBsAg and HBcAg are expressed separately from another CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at two splicing acceptor sites, SA<sub>1</sub> and SA<sub>2</sub>. The shuttle vector produced is designated pLAd-CMV-SD/SA<sub>1</sub>-HBsAg-SA<sub>2</sub>-HbcAg.

Sequences encoding multiple immuno-stimulators, including IL-2 (with a silent mutation caused by deletion of Xba I site), INF- $\gamma$ , and GMCSF, were inserted into E4 region of the adenoviral genome using the shuttle vector pRAd (**Figure 4A**, right side). These three immuno-stimulators are expressed separately from another CMV promoter via a retroviral splicing donor (SD) and acceptor (SA) mechanism at three splicing acceptor sites, SA<sub>1</sub>, SA<sub>2</sub>, and SA<sub>3</sub>. The shuttle vector produced is designated pRAd-CMV-SD/SA<sub>1</sub>-IL2-SA<sub>2</sub>-INF $\gamma$ -SA<sub>3</sub>-GMCSF.

Both pLAd-CMV-SD/SA<sub>1</sub>-HBsAg-SA<sub>2</sub>-HbcAg and pRAd-CMV-SD/SA<sub>1</sub>-IL2-SA<sub>2</sub>-INF $\gamma$ -SA<sub>3</sub>-GMCSF were linearized using appropriate restriction enzymes such as Xba I and EcoRI and ligated to the backbone of the adenovirus (**Figure 4B**), resulting in the recombinant adenoviral vector designated Ad.HBcAg.IL2.

2) Immune responses of animals to the adenoviral vaccines against HBV antigens

30

Experimental mice were inoculated with the adoviral vaccine constructed above, Ad.HBsAg.IL2 and Ad.HBcAg.IL2, to elicit immune response to the hepatitis B surface antigen and core antigen expressed by these two vectors, respectively. Immunogenicity of these adenoviral vectors

was determined by measuring titers of antibodies against HBsAg and HbcAg, respectively.

a) *HBV surface antigen (HBsAg) antibody titers*

5 CD-1 mice (Charles River Laboratories, Wilmington, MA) were injected intramuscularly with several different concentrations of Ad.HBsAg.IL2:  $10^0$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $5 \times 10^5$ ,  $5 \times 10^6$ , and  $5 \times 10^7$  pfu virus. **Figure 10A** shows the relative Anti-HBsAg antibody titers measured for sera harvested from mice inoculated with  $1 \times 10^5$  and  $5 \times 10^5$  pfu. Serum in each measurement was  
10 diluted 1:500. **Figure 10B** shows the relative Anti-HbsAg antibody titers measured for sera harvested from mice inoculated with  $1 \times 10^7$  and  $1 \times 10^8$  pfu. Serum in each measurement was diluted 1:1500.

To measure the relative titers of the Anti-HbsAg antibody elicited by Ad.HBsAg.IL2, blood (about 150-500  $\mu$ l) from each animal was collected from  
15 immunized mice every two weeks and serum was prepared. Blood was incubated at room temperature for 2-3 h to allow for clotting. The blood was then chilled overnight at 4°C to shrink the clot. Unclothed liquid was transferred to a clean tube and centrifuged at 2000 x g for 5 min. The  
supernatant was transferred to another clean tube. Sodium azide ( $\text{NaN}_3$ ) was  
20 added to 0.05% as a preservative. Small aliquots were kept at 4°C for short-term storage. Long-term storage was at -80°C.

Relative anti-HBsAg titers were determined by ELISA against recombinant HBsAg purified from yeast (from Aldevron, LLC, Fargo, ND). As shown in **Figure 10A**, the mice in group 1 had increasingly strong immune  
25 responses to HBsAg expressed by the adenoviral vector, Ad.HBsAg.IL2, within 8 weeks post inoculation. This vector with a titer as low as  $5 \times 10^5$  pfu was sufficient to elicit high levels of antibody specifically against HBsAg.

**Figure 10B** shows the immunogenicity of Ad.HBsAg.IL2 with higher titers. As shown in **Figure 10B**, immunogenicity of Ad.HBsAg.IL2 increased  
30 dramatically as the titer of the adenoviral vector was increased from  $1 \times 10^7$  pfu to  $1 \times 10^8$  pfu.

These results demonstrate that the adenoviral vector expressing both hepatitis B surface antigen and IL-2 can induce strong immune response specifically targeting the viral antigen in mice inoculated with this vector.  
35 These results also support the belief that the genetic vaccines provided by the

present invention hold great promises as efficacious vaccines for humans against hepatitis B virus.

*b) HBV core antigen (HBcAg) antibody titers*

5           Groups of C57BL/6 mice (Charles River Laboratories, Wilmington, MA) were injected intramuscularly with  $1 \times 10^7$  pfu Ad.HBcAg.IL2 on different dates. Blood was collected from four animals every two weeks following inoculation and serum was prepared. At 91 days (Group 3, **Figure 11A**) or 84 days (Group 4, **Figure 11B**) post-inoculation, mice were re-challenged with an  
10       additional  $1 \times 10^7$  pfu virus. Blood was collected from three animals every day following secondary challenge. Antibody titer was determined by ELISA against recombinant HBcAg purified from *E. coli* (from Chemicon International, Inc., Temecula, CA).

          As shown in **Figure 11A**, mice in group 3 had strong immune response  
15       to the hepatitis core antigen HBcAg expressed by the adenoviral vector Ad.HBcAg.IL2, with the highest titer of antibody against HBcAg reached in about 28 days post inoculation. The second inoculation with Ad.HBcAg.IL2 boosted the immune response again and very high titers were achieved within about 3 days of the second inoculation.

20           As shown in **Figure 11B**, mice in group 4 also had strong immune response to the hepatitis core antigen HBcAg expressed by the adenoviral vector Ad.HBcAg.IL2, with the high titer of antibody against HBcAg reached in about 34 days post inoculation. The second inoculation with Ad.HBcAg.IL2 boosted the immune response again and very high titers were achieved within  
25       about 3 days of the second inoculation.

          These results demonstrate that the adenoviral vector expressing both hepatitis B core antigen and IL-2 can also induce strong immune response specifically targeting the viral antigen in mice inoculated with this vector. These results once again support the belief that the genetic vaccines provided  
30       by the present invention hold great promises as efficacious vaccines for humans against hepatitis B virus.

**CLAIMS**

What is claimed is:

- 5       1.       A recombinant adenovirus comprising:  
          an HIV sequence encoding an HIV antigen, expression of the HIV  
          antigen by the recombinant adenovirus eliciting an immune response directed  
          against the HIV antigen in a host upon infection of the host by the  
          recombinant adenovirus.
- 10       2.       The recombinant adenovirus of claim 1, wherein the recombinant  
          adenovirus is replication-incompetent.
3.       The recombinant adenovirus of claim 1, wherein the HIV antigen is an  
15       antigen of HIV-1 or HIV-2.
4.       The recombinant adenovirus of claim 1, wherein the HIV antigen is an  
          antigen of HIV strain BH10 or pNL4-3.
- 20       5.       The recombinant adenovirus of claim 1, wherein the HIV antigen is an  
          antigen of HIV clade A, B, C, D, E, F, or G.
6.       The recombinant adenovirus of claim 1, wherein the HIV antigen is an  
          HIV glycoprotein or surface antigen.
- 25       7.       The recombinant adenovirus of claim 6, wherein the HIV glycoprotein  
          is an HIV envelope protein.
8.       The recombinant adenovirus of claim 7, wherein the HIV envelope  
30       protein is a wild type or mutant gp160, gp120, or gp41.
9.       The recombinant adenovirus of claim 7, wherein the cleavage site of  
          the HIV envelope protein is inactivated by mutation.

10. The recombinant adenovirus of claim 7, wherein the C-terminal cytosolic domain of the HIV envelope protein is deleted.
- 5 11. The recombinant adenovirus of claim 7, wherein both the cleavage site and the C-terminal cytosolic domain of the HIV envelope protein are deleted.
12. The recombinant adenovirus of claim 7, wherein the HIV envelop protein is encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 14, 16, 20, 21, 22, 23, and 24.
- 10 13. The recombinant adenovirus of claim 7, wherein the HIV sequence further encodes an HIV protein selected from the group consisting of RT, PR, Tat, Vif, Nef, and Rev.
- 15 14. The recombinant adenovirus of claim 7, wherein the HIV antigen is a modified HIV envelope protein that includes multiclade variable loops.
15. The recombinant adenovirus of claim 14, wherein the multiclade variable loops are V3 loops from at least two HIV clades.
- 20 16. The recombinant adenovirus of claim 15, wherein the at least two HIV clades are selected from the group consisting of clade A, B, C, D, E, F, and G of group M of HIV-1 isolates.
- 25 17. The recombinant adenovirus of claim 15, wherein the V3 loops are encoded by polynucleotides selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29, 30, and 31.
- 30 18. The recombinant adenovirus of claim 14, wherein the modified HIV envelope protein that includes multiclade variable loops is encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 32, 52, and 54.
19. The recombinant adenovirus of claim 1, further comprising:

a polynucleotide encoding a signal peptide that facilitates the secretion of the HIV antigen by a cell infected by the recombinant adenoviruse.

20. The recombinant adenovirus of claim 19, wherein the signal peptide is  
5 an HIV gp120 signal peptide.

21. The recombinant adenovirus of claim 19, wherein the signal peptide is encoded by SEQ ID NO: 74.

10 22. The recombinant adenovirus of claim 1, further comprising:  
a polynucleotide encoding an membrane-anchoring domain that renders the HIV antigen bound to the surface of a cell infected by the recombinant adenoviruse.

15 23. The recombinant adenovirus of claim 22, wherein the membrane-anchoring domain is an HIV gp41 transmembrane domain.

24. The recombinant adenovirus of claim 22, wherein the membrane-anchoring domain is encoded by SEQ ID NO: 75.

20 25. The recombinant adenovirus of claim 1, wherein the HIV antigen is an HIV structural protein.

26. The recombinant adenovirus of claim 25, wherein the HIV structural  
25 protein is a wild type HIV Gag.

27. The recombinant adenovirus of claim 25, wherein the HIV structural protein is a proteolytic fragment of HIV Gag.

30 28. The recombinant adenovirus of claim 27, wherein the proteolytic fragment of HIV Gag is selected from the group consisting of p17/24, p17 and p24.

29. The recombinant adenovirus of claim 27, wherein the proteolytic  
35 fragment of HIV Gag is in a natural, secreted or membrane bound form.

30. The recombinant adenovirus of claim 27, wherein the proteolytic fragment of Gag is encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 34, 35, 36, 40, 41, 42, 46, 47, and 48.
- 5 31. The recombinant adenovirus of claim 1, further comprising:  
a polynucleotide encoding an HIV protease or an HIV reverse transcriptase.
- 10 32. The recombinant adenovirus of claim 31, wherein the polynucleotide encoding an HIV protease is SEQ ID NO: 56.
33. The recombinant adenovirus of claim 31, wherein the HIV antigen is HIV Gag.
- 15 34. The recombinant adenovirus of claim 33, wherein the protease is expressed as a fusion protein with the HIV Gag.
35. The recombinant adenovirus of claim 33, wherein the protease is expressed separately from a promoter different from that for the HIV Gag.
- 20 36. The recombinant adenovirus of claim 33, wherein the protease is expressed as a separate protein from the same promoter for the HIV Gag via an IRES or splicing donor/acceptor mechanism.
- 25 37. The recombinant adenovirus of claim 1, further comprising:  
a polynucleotide encoding a fusion protein of HIV protease and HIV reverse transcriptase.
- 30 38. The recombinant adenovirus of claim 37, wherein the HIV antigen is HIV Gag.
- 35 39. The recombinant adenovirus of claim 38, wherein the fusion protein of HIV protease and HIV reverse transcriptase is expressed as a fusion protein with the HIV Gag.



40. The recombinant adenovirus of claim 38, wherein the fusion protein of HIV protease and HIV reverse transcriptase is expressed separately from a promoter different from that for the HIV Gag.
- 5
41. The recombinant adenovirus of claim 38, wherein the fusion protein of HIV protease and HIV reverse transcriptase is expressed as a separate protein from the same promoter for the HIV Gag via an IRES or splicing donor/acceptor mechanism.
- 10
42. The recombinant adenovirus of claim 1, further comprising:  
a polynucleotide encoding a fusion protein of HIV protease, HIV reverse transcriptase and HIV integrase.
- 15
43. The recombinant adenovirus of claim 42, wherein the HIV antigen is HIV Gag.
- 20
44. The recombinant adenovirus of claim 43, wherein the fusion protein of HIV protease, HIV reverse transcriptase and HIV integrase is expressed as a fusion protein with the HIV Gag.
- 25
45. The recombinant adenovirus of claim 43, wherein the fusion protein of HIV protease, HIV reverse transcriptase and HIV integrase is expressed separately from a promoter different from that for the HIV Gag.
- 30
46. The recombinant adenovirus of claim 43, wherein the fusion protein of HIV protease, HIV reverse transcriptase and HIV integrase is expressed as a separate protein from the same promoter for the HIV Gag via an IRES or splicing donor/acceptor mechanism.
47. The recombinant adenovirus of claim 1, further comprising:  
an immuno-stimulator sequence heterologous to adenovirus and encoding an immuno-stimulator whose expression in the host enhances the immunogenicity of the HIV antigen.

48. The recombinant adenovirus of claim 47, wherein the HIV sequence is positioned in the E1 region of the adenovirus and the immuno-stimulator sequence is positioned in the E4 region of the adenovirus.
- 5 49. The recombinant adenovirus of claim 47, wherein  
both the HIV sequence and the immuno-stimulator sequence are  
positioned in the E1 or E4 region of the adenovirus, and are expressed from  
the same promoter bicistronically via an internal ribosomal entry site or via a  
splicing donor-acceptor mechanism.
- 10 50. The recombinant adenovirus of claim 47, wherein the expression of the  
HIV antigen or the immuno-stimulator is controlled by an adenoviral promoter.
- 15 51. The recombinant adenovirus of claim 47, wherein the expression of the  
HIV antigen or the immuno-stimulator is controlled by a non-adenoviral  
promoter.
- 20 52. The recombinant adenovirus of claim 51, wherein the non-adenoviral  
promoter is selected from the group consisting of CMV promoter, SV40  
promoter, retrovirus LTR promoter, and chicken cytoplasmic  $\beta$ -actin promoter.
53. The recombinant adenovirus of claim 47, wherein the immuno-  
stimulator is a cytokine.
- 25 54. The recombinant adenovirus of claim 53, wherein the cytokine is  
selected from the group consisting of interleukin-2, interleukin-4, interleukin-  
12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating  
factor, and granulocyte-macrophage colony stimulating factor.
- 30 55. The recombinant adenovirus of claim 47, wherein the immuno-  
stimulator is a combination of different cytokines.
- 35 56. The recombinant adenovirus of claim 55, wherein the combination of  
cytokines are expressed from the same promoter but as separate proteins via  
an IRES mechanism or a retroviral splicing donor/acceptor mechanism.

57. A recombinant adenovirus comprising:  
a first HIV sequence encoding a first HIV antigen, expression of which  
is under the transcriptional control of a first promoter; and  
5 a second HIV sequence encoding a second HIV antigen, expression of  
which is under the transcriptional control of a second promoter positioned in a  
different region than the first promoter,  
expression of the first and second HIV sequences eliciting an immune  
response directed against the first and second HIV antigens upon infection of  
10 the host by the recombinant virus.
58. The recombinant adenovirus of claim 57, wherein the recombinant  
adenovirus is replication-incompetent.
- 15 59. The recombinant adenovirus of claim 57, wherein the first and second  
HIV antigens are the same.
60. The recombinant adenovirus of claim 57, wherein the first and second  
HIV antigens are different.  
20
61. The recombinant adenovirus of claim 57, wherein the first or second  
HIV antigen is an HIV envelope protein.
62. The recombinant adenovirus of claim 61, wherein the HIV envelope  
25 protein is a wild type or mutant gp160, gp120, or gp41.
63. The recombinant adenovirus of claim 62, wherein the cleavage site of  
the HIV envelope protein is inactivated by mutation.
- 30 64. The recombinant adenovirus of claim 62, wherein the C-terminal  
cytosolic domain of the HIV envelope protein is deleted.
65. The recombinant adenovirus of claim 62, wherein both the cleavage  
site and the C-terminal cytosolic domain of the HIV envelope protein are  
35 deleted.

66. The recombinant adenovirus of claim 61, wherein the first or second HIV sequence further encodes an HIV protein selected from the group consisting of RT, PR, Tat, Vif, Nef, and Rev.
- 5 67. The recombinant adenovirus of claim 57, wherein the first or second HIV antigen is a modified HIV envelope protein that includes multiclade variable loops.
- 10 68. The recombinant adenovirus of claim 67, wherein the multiclade variable loops are V3 loops from at least two HIV clades.
69. The recombinant adenovirus of claim 68, wherein the at least two HIV clades are selected from the group consisting of clade A, B, C, D, E, F, and G of group M of HIV-1 isolates.
- 15 70. The recombinant adenovirus of claim 68, wherein the V3 loops are encoded by polynucleotides selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, 29, 30, and 31.
- 20 71. The recombinant adenovirus of claim 57, further comprising:  
a polynucleotide encoding a signal peptide that facilitates the secretion of the first or second HIV antigen by a cell infected by the recombinant adenoviruse.
- 25 72. The recombinant adenovirus of claim 71, wherein the signal peptide is an HIV gp120 signal peptide.
73. The recombinant adenovirus of claim 71, wherein the signal peptide is encoded by SEQ ID NO: 74.
- 30 74. The recombinant adenovirus of claim 57, further comprising:  
a polynucleotide encoding an membrane-anchoring domain that renders the first or second HIV antigen bound to the surface of a cell infected by the recombinant adenoviruse.
- 35

75. The recombinant adenovirus of claim 74, wherein the membrane-anchoring domain is an HIV gp41 transmembrane domain.
- 5 76. The recombinant adenovirus of claim 74, wherein the membrane-anchoring domain is encoded by SEQ ID NO: 75.
77. The recombinant adenovirus of claim 57, wherein the first and second HIV antigen is an HIV structural protein.
- 10 78. The recombinant adenovirus of claim 77, wherein the HIV structural protein is a wild type HIV Gag.
79. The recombinant adenovirus of claim 77, wherein the HIV structural protein is a proteolytic fragment of HIV Gag.
- 15 80. The recombinant adenovirus of claim 77, wherein the proteolytic fragment of HIV Gag is selected from the group consisting of p17/24, p17 and p24.
- 20 81. The recombinant adenovirus of claim 77, wherein the proteolytic fragment of HIV Gag is in a natural, secreted or membrane bound form.
82. The recombinant adenovirus of claim 77, wherein the proteolytic fragment of Gag is encoded by a polynucleotide selected from the group consisting of SEQ ID NOs: 34, 35, 36, 40, 41, 42, 46, 47, and 48.
- 25 83. The recombinant adenovirus of claim 77, further comprising:  
a polynucleotide encoding an HIV protease.
- 30 84. The recombinant adenovirus of claim 83, wherein the polynucleotide encoding an HIV protease is SEQ ID NO: 56.

85. The recombinant adenovirus of claim 57, wherein the first HIV antigen is a wildtype or mutant HIV envelope protein, and the second HIV antigen is a wildtype or mutant HIV structural protein.
- 5 86. The recombinant adenovirus of claim 85, wherein wildtype or mutant HIV structural protein is wildtype Gag or a proteolytic fragment of Gag.
87. The recombinant adenovirus of claim 57, wherein both the first and second HIV antigen are a wildtype or mutant HIV envelope protein.
- 10 88. The recombinant adenovirus of claim 57, wherein both the first and second HIV antigen are a wildtype or mutant HIV structural protein.
89. The recombinant adenovirus of claim 57, further comprising:  
15 an immuno-stimulator sequence heterologous to adenovirus and encoding an immuno-stimulator whose expression in the host enhances the immunogenicity of the first or second HIV antigen.
90. The recombinant adenovirus of claim 89, wherein the first or second  
20 HIV sequence and the immuno-stimulator sequence are expressed from the same promoter bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.
91. The recombinant adenovirus of claim 89, wherein the immuno-  
25 stimulator is a cytokine.
92. The recombinant adenovirus of claim 91, wherein the cytokine is selected from the group consisting of interleukin-2, interleukin-4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor, and granulocyte-macrophage colony stimulating factor.
- 30 93. The recombinant adenovirus of claim 57, wherein the first or second promoter is an adenoviral promoter.

94. The recombinant adenovirus of claim 57, wherein the first or second promoter is non-adenoviral promoter.
95. The recombinant adenovirus of claim 94, wherein the non-adenoviral promoter is selected from the group consisting of CMV promoter, SV40 promoter, retrovirus LTR promoter, and chicken cytoplasmic  $\beta$ -actin promoter.
96. The recombinant adenovirus of claim 57, wherein the first promoter is in the E1 region of the adenovirus and the second promoter is positioned in the E4 region of the adenovirus.
97. A method for enhancing the immunity of a host to HIV infection, comprising:  
administering to the host a first recombinant adenovirus comprising a first HIV sequence encoding a first HIV antigen, expression of the first HIV antigen by the first recombinant adenovirus eliciting an immune response directed against the first HIV antigen in a host upon infection of the host by the recombinant adenovirus.
98. The method of claim 97, wherein administering to the host a recombinant adenovirus is performed intramuscularly, intratracheally, subcutaneously, intranasally, intradermally, rectally, orally or parentally.
99. The method of claim 97, wherein the recombinant adenovirus further comprises one or more immuno-stimulator sequences heterologous to adenovirus that encodes an immuno-stimulator whose expression in the host enhances the immunogenicity of the HIV antigen.
100. The method of claim 97, further comprising:  
administering to the host an immuno-stimulator.
101. The method of claim 100, wherein the immuno-stimulator is a cytokine selected from the group consisting of interleukin-2, interleukin-4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor, and granulocyte-macrophage colony stimulating factor.

102. The method of claim 97, further comprising:  
administering to the host a second recombinant adenovirus of different  
serotype than that of the first recombinant adenovirus and comprising  
5 a second HIV sequence encoding a second HIV antigen, expression of  
the second HIV antigen by the second recombinant adenovirus eliciting an  
immune response directed against the second HIV antigen in a host upon  
infection of the host by the recombinant adenovirus.
- 10 103. The method of claim 102, wherein the serotype of the first recombinant  
adenovirus is adenovirus serotype 5, and the serotype of the second  
recombinant adenovirus is selected from the group consisting of adenovirus  
serotype 1-4 and 6-51.
- 15 104. The method of claim 102, wherein the first HIV antigen encoded by the  
first recombinant adenovirus is the same as the second HIV antigen encoded  
by the second recombinant adenovirus.
- 20 105. The method of claim 102, wherein the first HIV antigen encoded by the  
first recombinant adenovirus is different from the second HIV antigen encoded  
by the second recombinant adenovirus.
- 25 106. The method of claim 102, wherein the second recombinant adenovirus  
has the same adenoviral backbone as the first one except that the fiber region  
of the second recombinant adenovirus is of different serotype than that in the  
first recombinant adenovirus.
- 30 107. The method of claim 102, wherein the second recombinant adenovirus  
has the same adenoviral backbone as the first one except that the knob,  
shaft, or penton base domain of the fiber region of the second recombinant  
adenovirus is of different serotype than the corresponding one in the first  
recombinant adenovirus.



108. The method of claim 102, wherein the second recombinant adenovirus is administered to the host at least one week post the administration of the first recombinant adenovirus.
- 5 109. The method of claim 97, further comprising:  
harvesting serum from the host after the administration of the first recombinant adenovirus.
- 10 110. The method of claim 109, wherein the host is a human or a non-human primate.
111. The method of claim 109, further comprising:  
storing the serum for at least 12 hour; and  
then administering the serum to the host or another host.
- 15 112. The method of claim 111, wherein the other host is a human or a non-human primate.
113. The method of claim 97, further comprising:  
isolating antibody against the first HIV antigen from the host after the  
20 administration of the first recombinant adenovirus; and  
then administering the antibody to host or another host.
114. A method of enhancing the immunity of a host to HIV infection, comprising:  
25 administering to the host a recombinant adenovirus comprising  
a first HIV sequence encoding a first HIV antigen, expression of which is under the transcriptional control of a first promoter; and  
a second HIV sequence encoding a second HIV antigen, expression of which is under the transcriptional control of a second promoter positioned in a  
30 different region than the first promoter,  
expression of the first and second HIV sequences eliciting an immune response directed against the first and second HIV antigens upon infection of the host by the recombinant virus.
- 35 115. The method of claim 114, further comprising:

administering to the host the recombinant adenovirus at least once again after the initial administration of the recombinant adenovirus.

116. A method for enhancing the immunity of a host to infection of a first  
5 and second pathogenic virus, comprising:

administering to the host a first recombinant adenovirus comprising a first antigen sequence heterologous to native adenovirus and encoding a first viral antigen from the first pathogenic virus, expression of the first viral antigen by the first recombinant adenovirus eliciting an immune response directed  
10 against the first viral antigen in a host upon infection of the host by the first recombinant adenovirus; and

administering to the host a second recombinant adenovirus comprising a second antigen sequence heterologous to native adenovirus and encoding a second viral antigen from the second pathogenic virus, expression of the  
15 second viral antigen by the second recombinant adenovirus eliciting an immune response directed against the second viral antigen in a host upon infection of the host by the first recombinant adenovirus.

117. The method of claim 116, wherein administering to the host the first or  
20 second recombinant adenovirus is performed intramuscularly, intratracheally, subcutaneously, intranasally, intradermally, rectally, orally or parentally.

118. The method of claim 116, wherein the serotype of the first recombinant adenovirus is adenovirus serotype 5, and the serotype of the second  
25 recombinant adenovirus is selected from the group consisting of adenovirus serotype 1-4 and 6-51.

119. The method of claim 116, wherein the first viral antigen encoded by the first recombinant adenovirus is the same as the second viral antigen encoded  
30 by the second recombinant adenovirus.

120. The method of claim 116, wherein the first viral antigen encoded by the first recombinant adenovirus is different from the second viral antigen encoded by the second recombinant adenovirus.

35

121. The method of claim 116, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the fiber region of the second recombinant adenovirus is of different serotype than that in the first recombinant adenovirus.

5

122. The method of claim 116, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the knob, shaft, or penton base domain of the fiber region of the second recombinant adenovirus is of different serotype than the corresponding one in the first recombinant adenovirus.

10

123. The method of claim 116, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the knob domain of the fiber region of the second recombinant adenovirus is of different serotype than the corresponding one in the first recombinant adenovirus.

15

124. The method of claim 116, wherein the second recombinant adenovirus is administered to the host at least one week post the administration of the first recombinant adenovirus.

20

125. The method of claim 116, wherein the first or second recombinant adenovirus is replication-incompetent.

126. The method of claim 116, wherein the first recombinant virus further comprises a third antigen sequence heterologous to native adenovirus and encoding a third viral antigen from the first or second pathogenic virus.

25

127. The method of claim 126, wherein the first and third antigen sequences are positioned in the E1 and E3 or E4 region of the native progenitor of the first recombinant adenovirus, respectively.

30

128. The method of claim 126, wherein the first and third antigen sequences are expressed bicistronically by the same promoter.

129. The method of claim 128, wherein the first and second antigen sequences are expressed bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

5 130. The method of claim 116, wherein  
the first recombinant virus further comprises a third antigen sequence heterologous to native adenovirus and encoding a third viral antigen from the first or second pathogenic virus, and  
the second recombinant virus further comprises a fourth antigen  
10 sequence heterologous to native adenovirus and encoding a fourth viral antigen from the first or second pathogenic virus.

131. The method of claim 130, wherein the first and third antigen sequences are positioned in the E1 and E3 or E4 region of the native  
15 progenitor of the first recombinant adenovirus, respectively; and the second and fourth antigen sequence are positioned in the E1 and E3 or E4 region of the native progenitor of the second recombinant adenovirus, respectively.

132. The method of claim 131, wherein the first and third antigen  
20 sequences are expressed bicistronically by the same promoter, or the second and fourth antigen sequences are expressed bicistronically by the same promoter,

133. The method of claim 132, wherein the bicistronic expression is via an  
25 internal ribosomal entry site or via a splicing donor-acceptor mechanism.

134. The method of claim 116, wherein the first and the second pathogenic viruses are the same.

30 135. The method of claim 116, wherein the first and the second pathogenic viruses are of the same type but of different subtype or clade.

136. The method of claim 116, wherein the first and the second pathogenic  
35 viruses are different types of the same virus.

137. The method of claim 116, wherein the first and the second pathogenic viruses are different viruses.
138. The method of claim 116, wherein the first or second pathogenic virus  
5 is a human immunodeficiency virus.
139. The method of claim 138, wherein the first or second viral antigen is an HIV surface, core/capsid, regulatory, enzyme or accessory protein.
- 10 140. The method of claim 138, wherein the first or second viral antigen is selected from the group consisting of HIV gp120, gp41, Gag, p17, p24, p2, p7, p1, p6, Tat, Rev, PR, RT, IN, Vif, Vpr, Vpx, Vpu and Nef.
141. The method of claim 116, wherein the first or second pathogenic virus  
15 is influenza virus.
142. The method of claim 141, wherein the first or second viral antigen is a glycoprotein of the influenza virus.
- 20 143. The method of claim 142, wherein the first or second viral antigen is influenza glycoprotein HA1, HA2 or NA.
144. The method of claim 116, wherein the first or second pathogenic virus is Ebola virus.  
25
145. The method of claim 144, wherein the first or second viral antigen is an Ebola glycoprotein.
146. The method of claim 145, wherein the first or second viral antigen is  
30 Ebola GP1 or GP2 protein.
147. The method of claim 146, wherein the first or second viral antigen is an Ebola nucleocapsid protein.

148. The method of claim 116, wherein the first or second pathogenic virus is Marburg virus.
149. The method of claim 148, wherein the first or second viral antigen is a Marburg glycoprotein.
150. The method of claim 148, wherein the first or second viral antigen is a Marburg nucleocapsid protein.
151. The method of claim 116, wherein the first or second pathogenic virus is Arbovirus.
152. The method of claim 151, wherein the first or second viral antigen is Arbovirus glycoprotein.
153. The method of claim 116, wherein the first or second pathogenic virus is hepatitis virus.
154. The method of claim 153, wherein the hepatitis virus is hepatitis A, B, C, D or E virus.
155. The method of claim 153, wherein the first or second viral antigen is surface antigen or core protein of hepatitis B virus.
156. The method of claim 155, wherein the first or second viral antigen is SHBsAg, MHBsAg, or LHBsAg of hepatitis B virus.
157. The method of claim 153, wherein the first or second viral antigen is a surface antigen or core protein of hepatitis C virus.
158. The method of claim 157, wherein the first or second viral antigen is NS3, NS4 or NS5 antigen of hepatitis C virus.
159. The method of claim 116, wherein the first or second pathogenic virus is respiratory syncytial virus.

160. The method of claim 159, wherein the first or second viral antigen is a glycoprotein or a fusion protein of respiratory syncytial virus
161. The method of claim 116, wherein the first or second pathogenic virus is herpes simplex virus.
- 5 162. The method of claim 161, wherein the first or second pathogenic virus is herpes simplex virus type-1 or type-2.
- 10 163. The method of claim 161, wherein the first or second viral antigen is glycoprotein D from herpes simplex virus type-2.
164. The method of claim 116, wherein the first or second pathogenic virus is human papilloma virus.
- 15 165. The method of claim 164, wherein the first or second viral antigen is E6 or E7 of human papilloma virus.
166. The method of claim 116, wherein the first or second viral antigen is a full-length antigenic viral protein or a portion of the antigenic viral protein that contains the predominant antigen, neutralizing antigen, or epitope of the first or second pathogenic virus.
- 20 167. The method of claim 116, wherein the first or second viral antigen is a modified antigen that is mutated from a glycoprotein of the first or second pathogenic virus such that the first or second viral antigen is rendered non-functional as a viral component but retains its antigenicity.
- 25 168. The method of claim 167, wherein the modification of first or second viral antigen includes deletions in the proteolytic cleavage site of the glycoprotein, and duplications and rearrangement of immunosuppressive peptide regions of the glycoprotein.
- 30 169. The method of claim 116, wherein the first or second recombinant adenovirus further comprises:

an immuno-stimulator sequence that is heterologous to native adenovirus and encodes an immuno-stimulator.

5 170. The method of claim 169, wherein the immuno-stimulator is a cytokine.

171. The method of claim 170, wherein the cytokine is selected from the group consisting of interleukin-2, interleukin-4, interleukin-12,  $\beta$ -interferon,  $\lambda$ -interferon,  $\gamma$ -interferon, granulocyte colony stimulating factor, and granulocyte-macrophage colony stimulating factor.

10

172. The method of claim 116, further comprising:  
harvesting serum from the host after the administration of the first and second recombinant adenovirus.

15 173. The method of claim 172, wherein the host is a human or a non-human primate.

174. The method of claim 172, further comprising:  
storing the serum for at least 12 hour; and  
20 then administering the serum to the host or another host.

175. The method of claim 174, wherein the other host is a human or a non-human primate.

25 176. The method of claim 116, further comprising:  
isolating antibody against the first or second viral antigen from the host after the administration of the first and second recombinant adenovirus; and  
then administering the antibody to host or another host.

30 177. The method of claim 176, wherein the host or the other host is a human or a non-human primate.



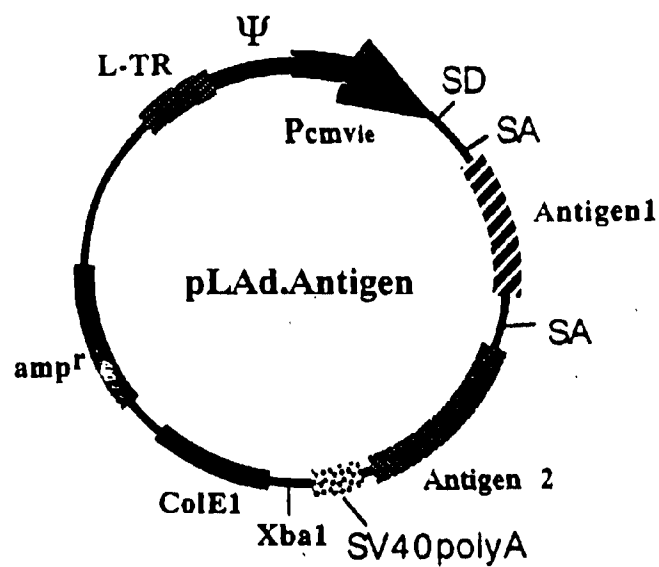
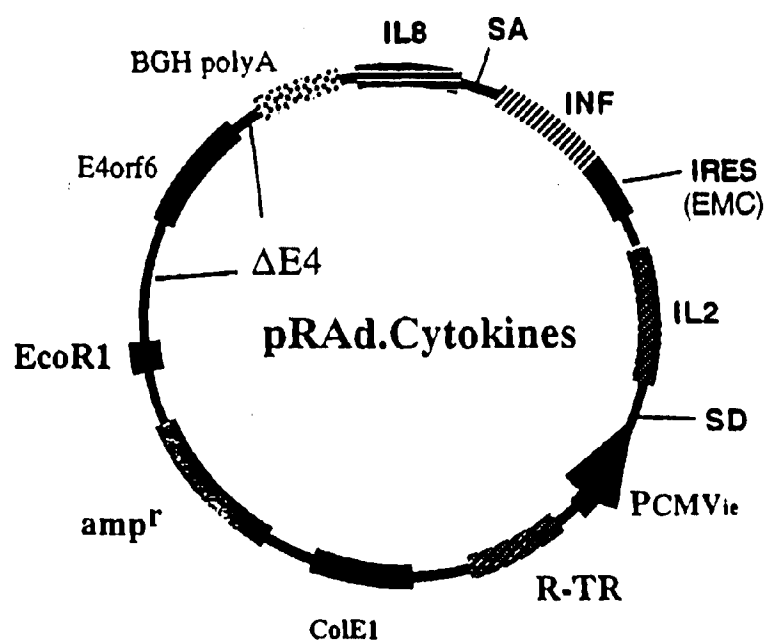
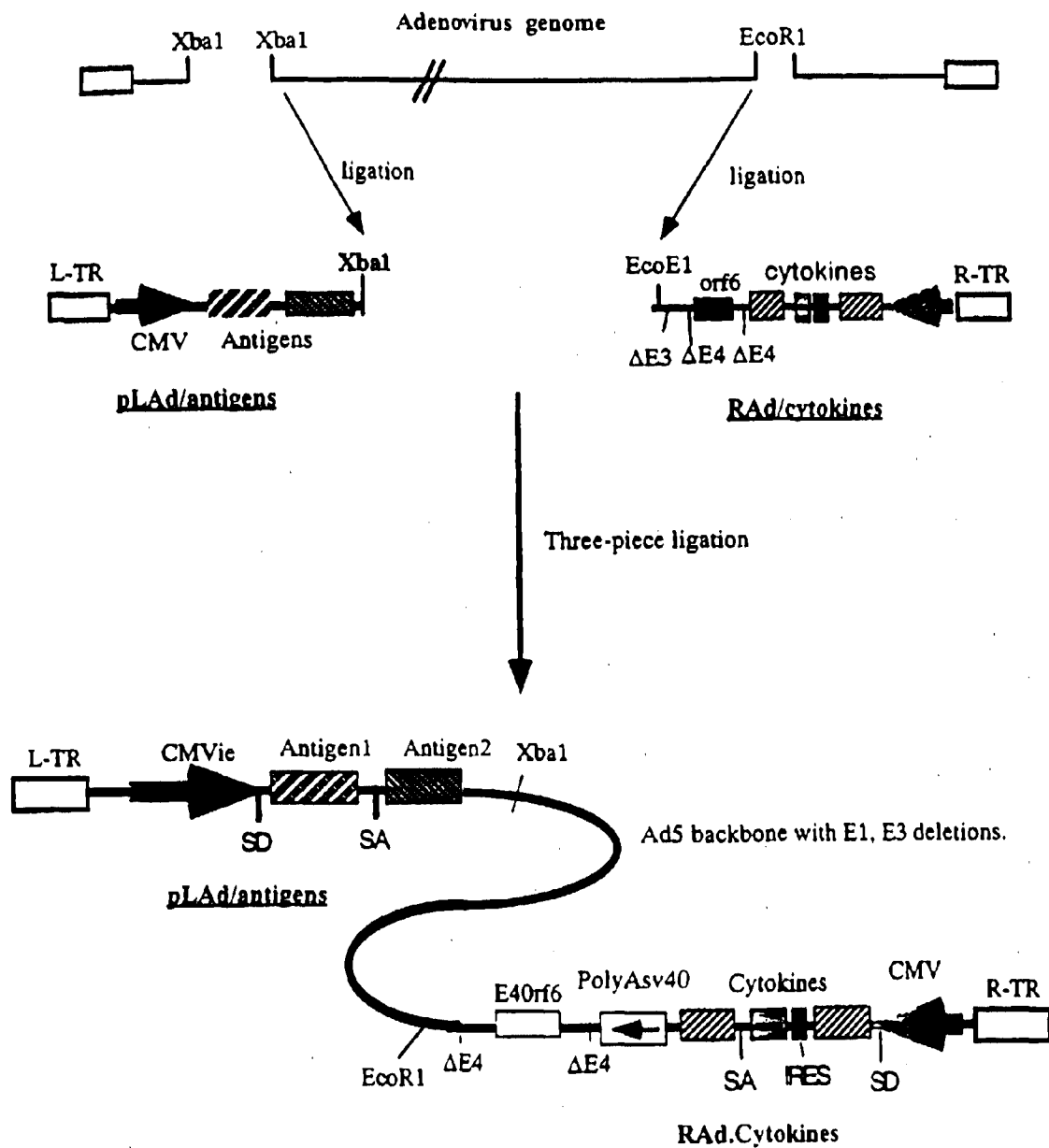
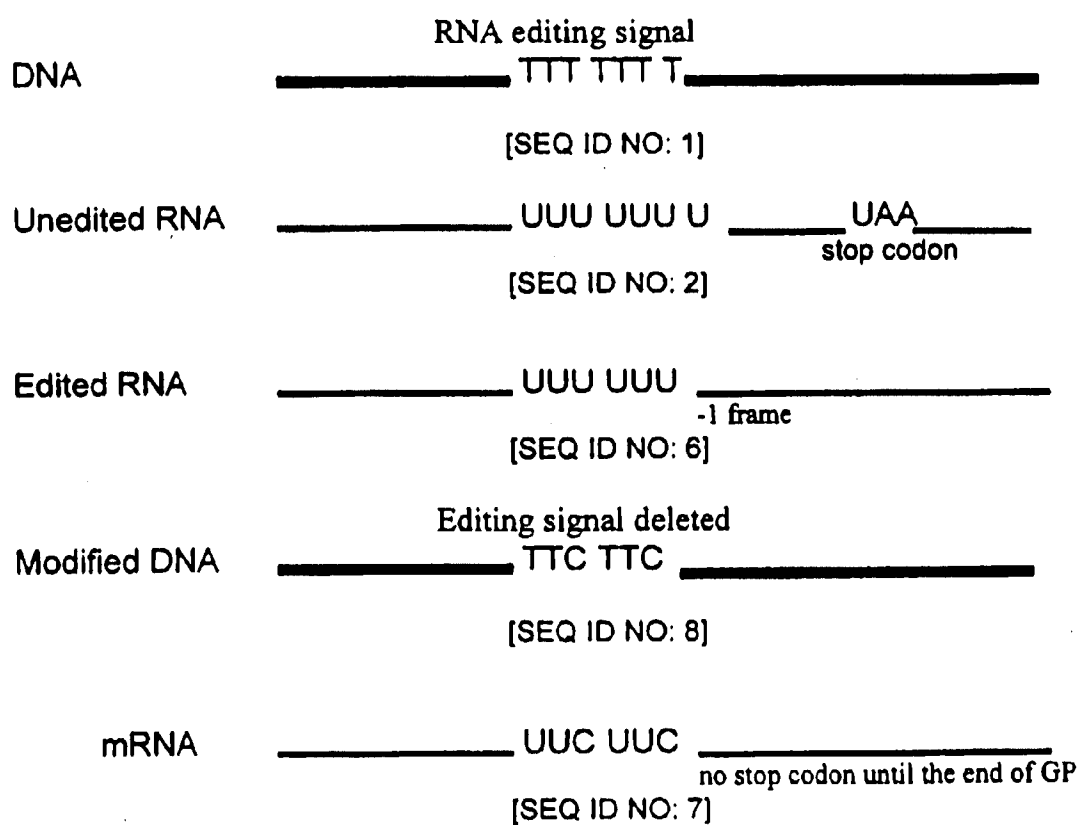
**FIGURE 1A****FIGURE 1B**

FIGURE 1C



**FIGURE 2**

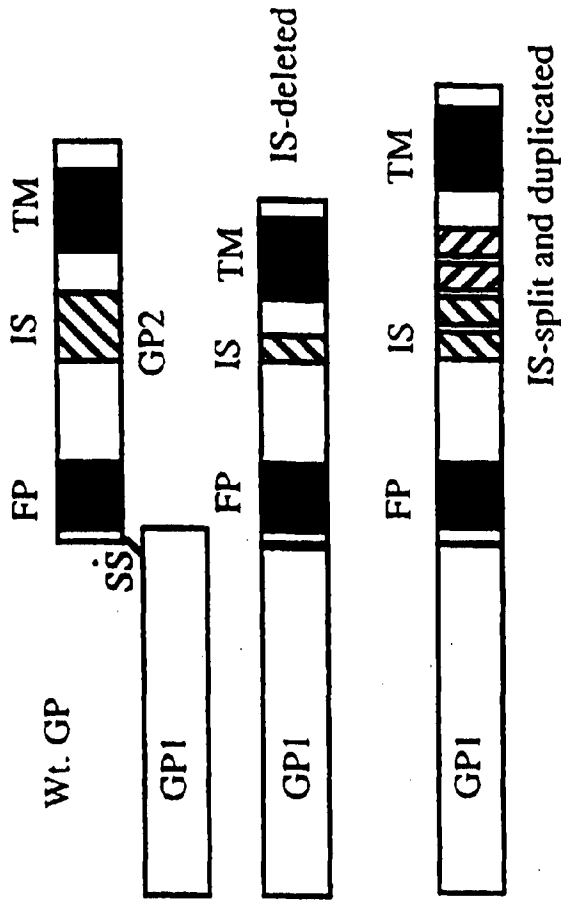
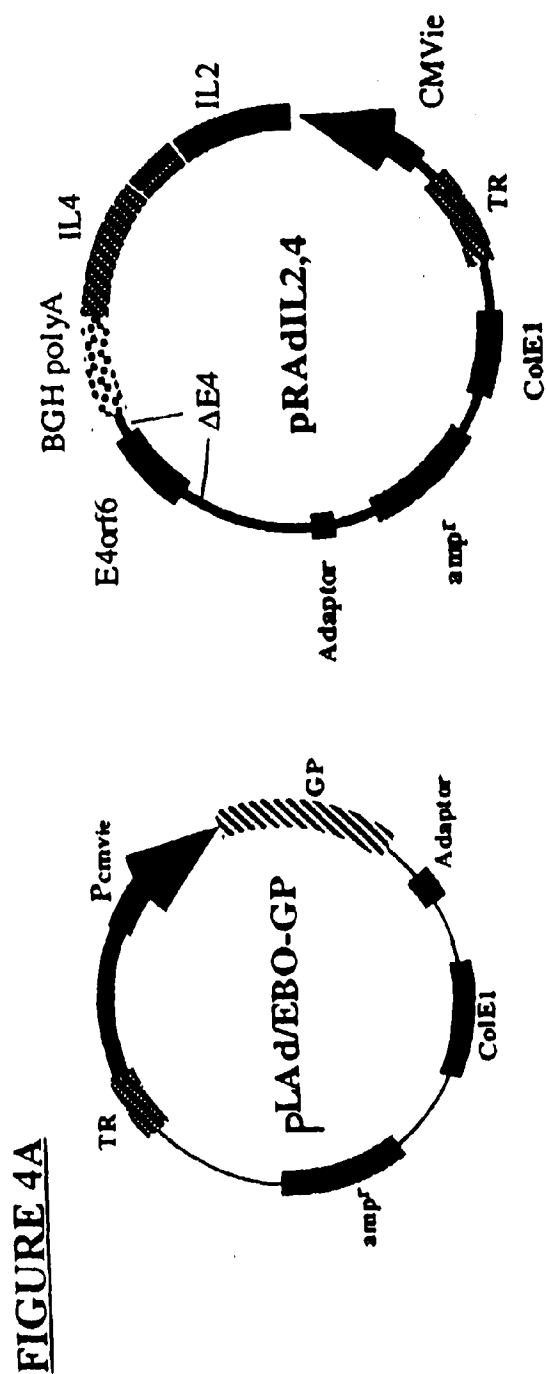


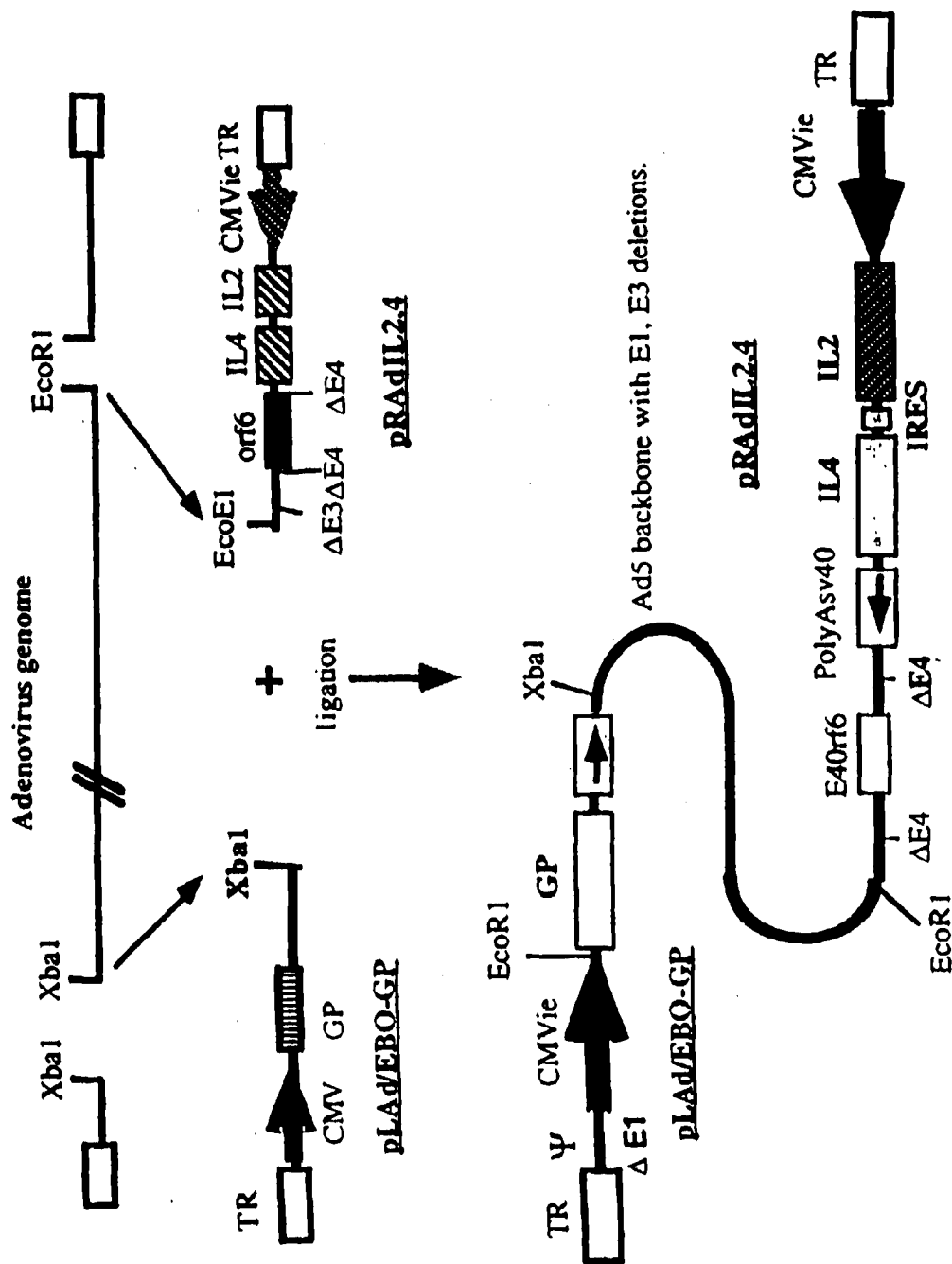
FIGURE 3A

FIGURE 3B

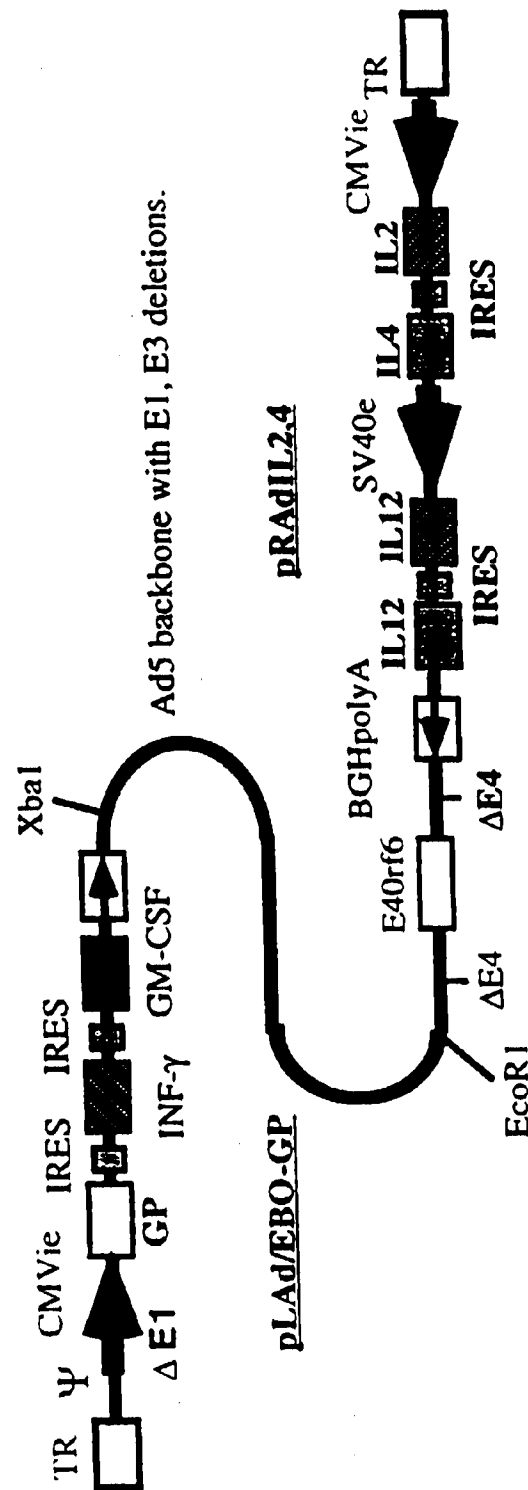
FIGURE 3C

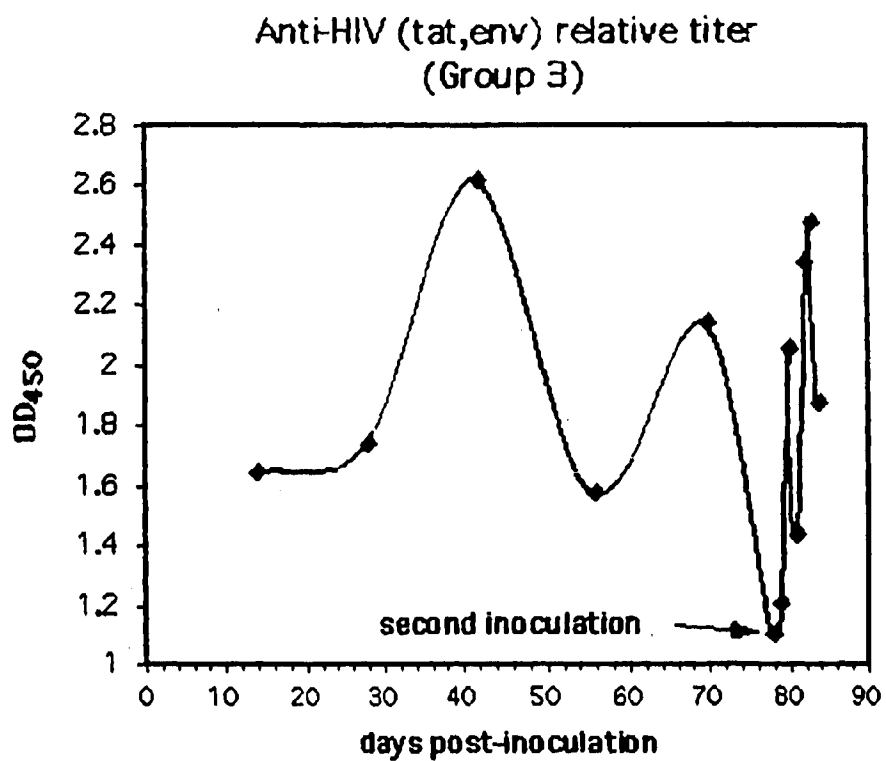


**FIGURE 4B**

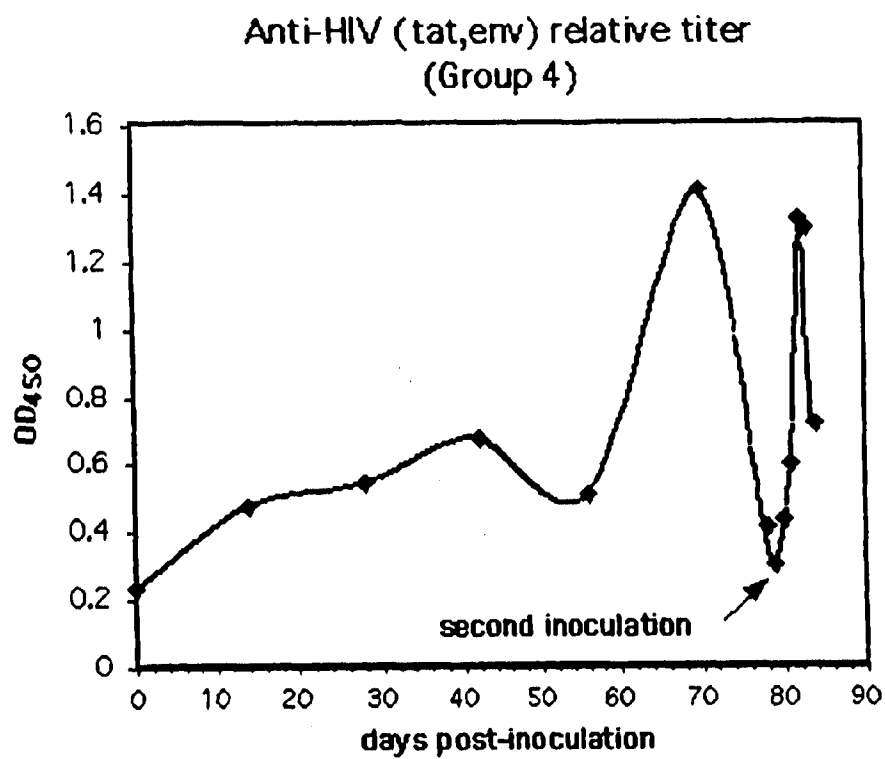


**FIGURE 5**



**FIGURE 6**



**FIGURE 7**

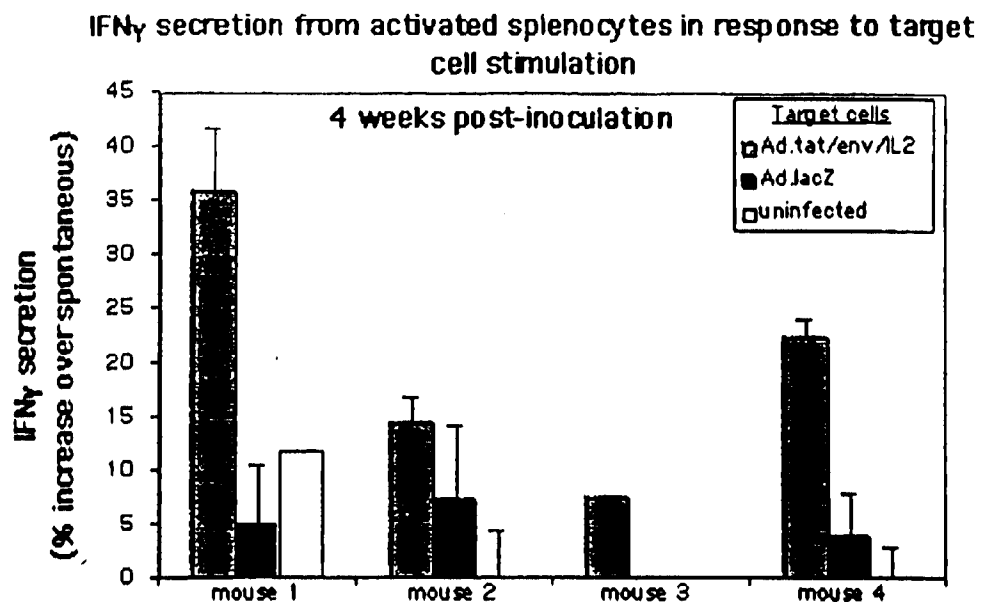
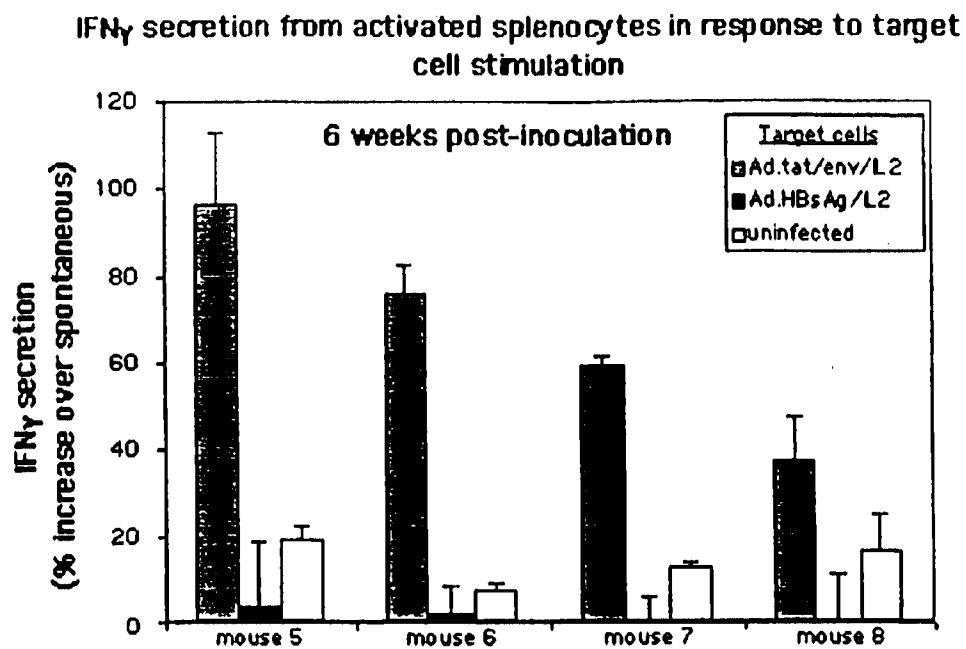
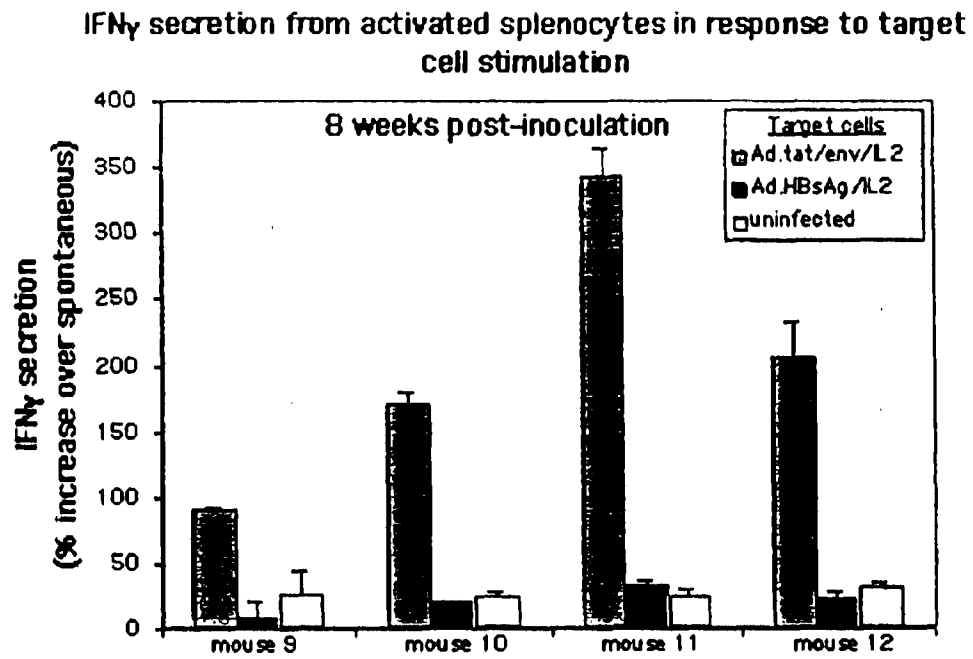
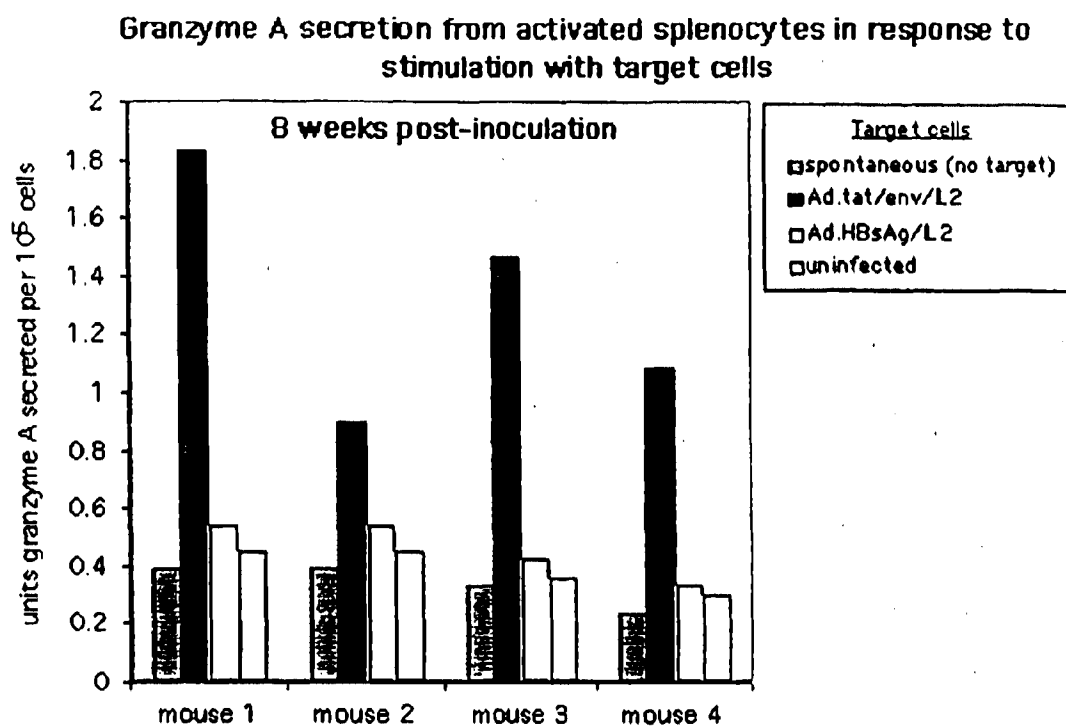
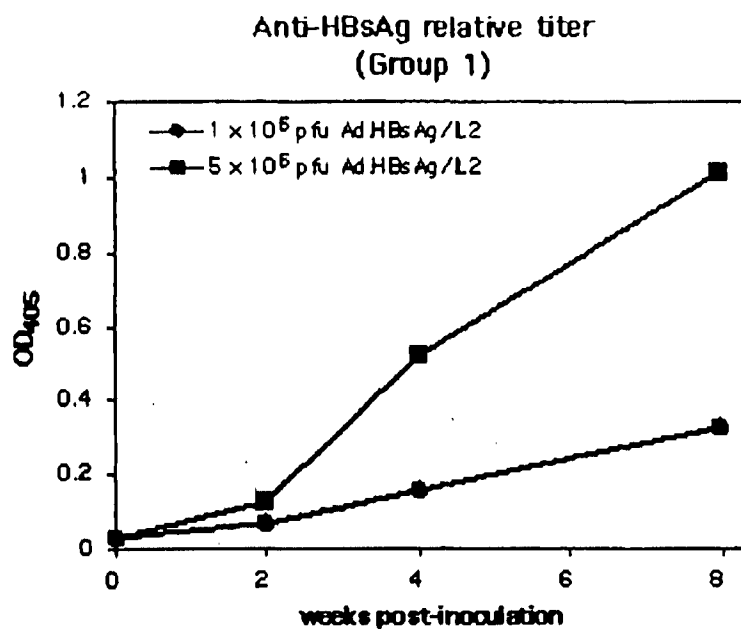
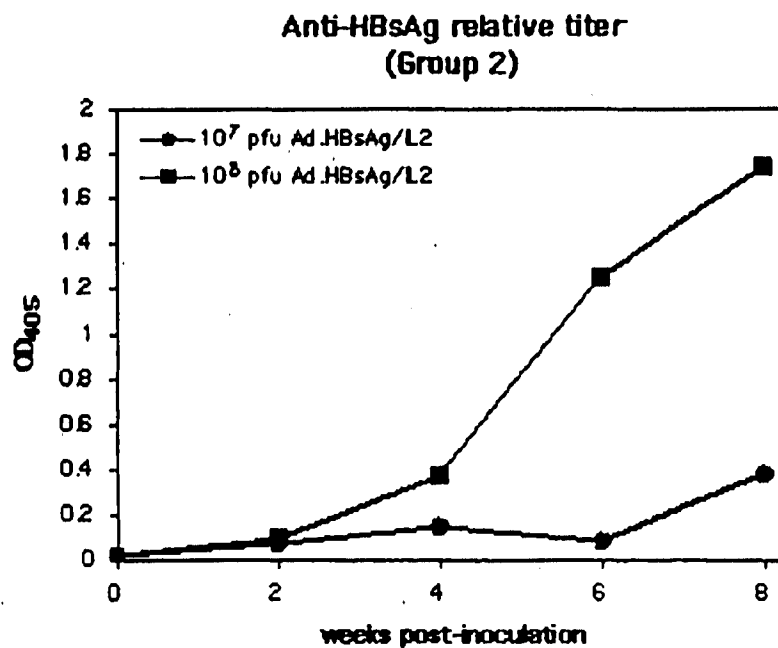


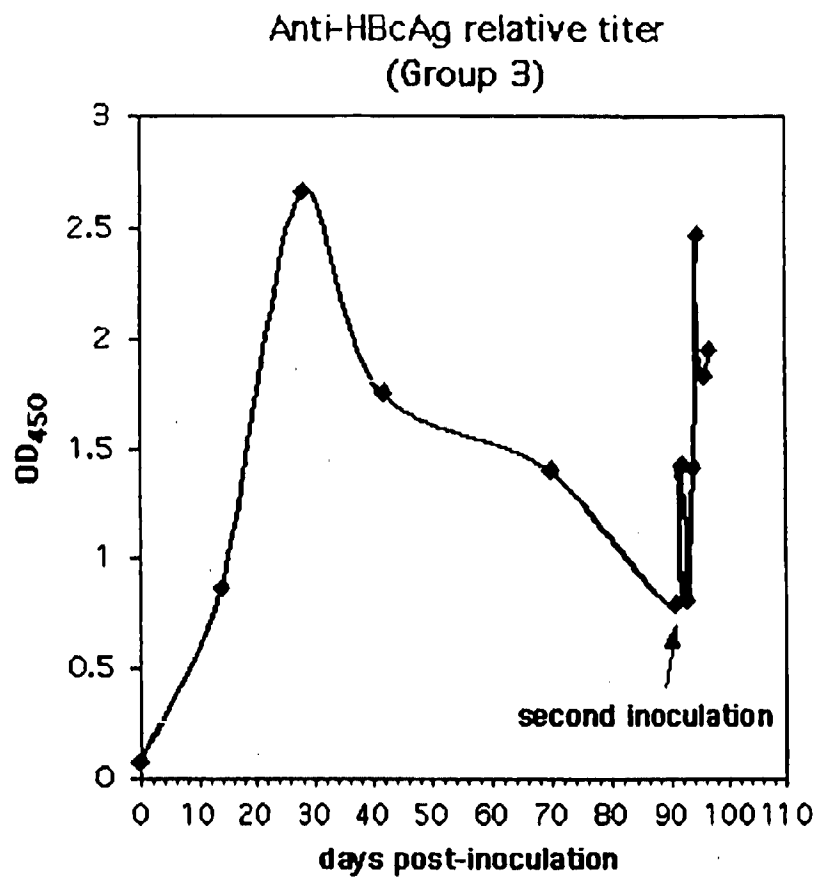
FIGURE 8A

**FIGURE 8B**

**FIGURE 8C**

**FIGURE 9**

**FIGURE 10A****FIGURE 10B**

**FIGURE 11A**

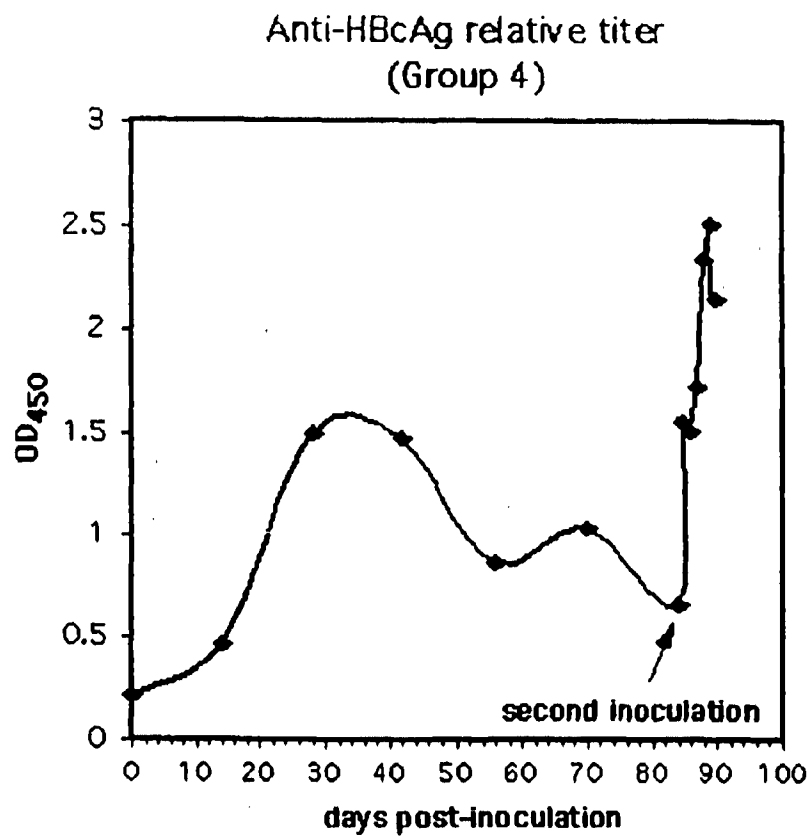
**FIGURE 11B**



FIGURE 12

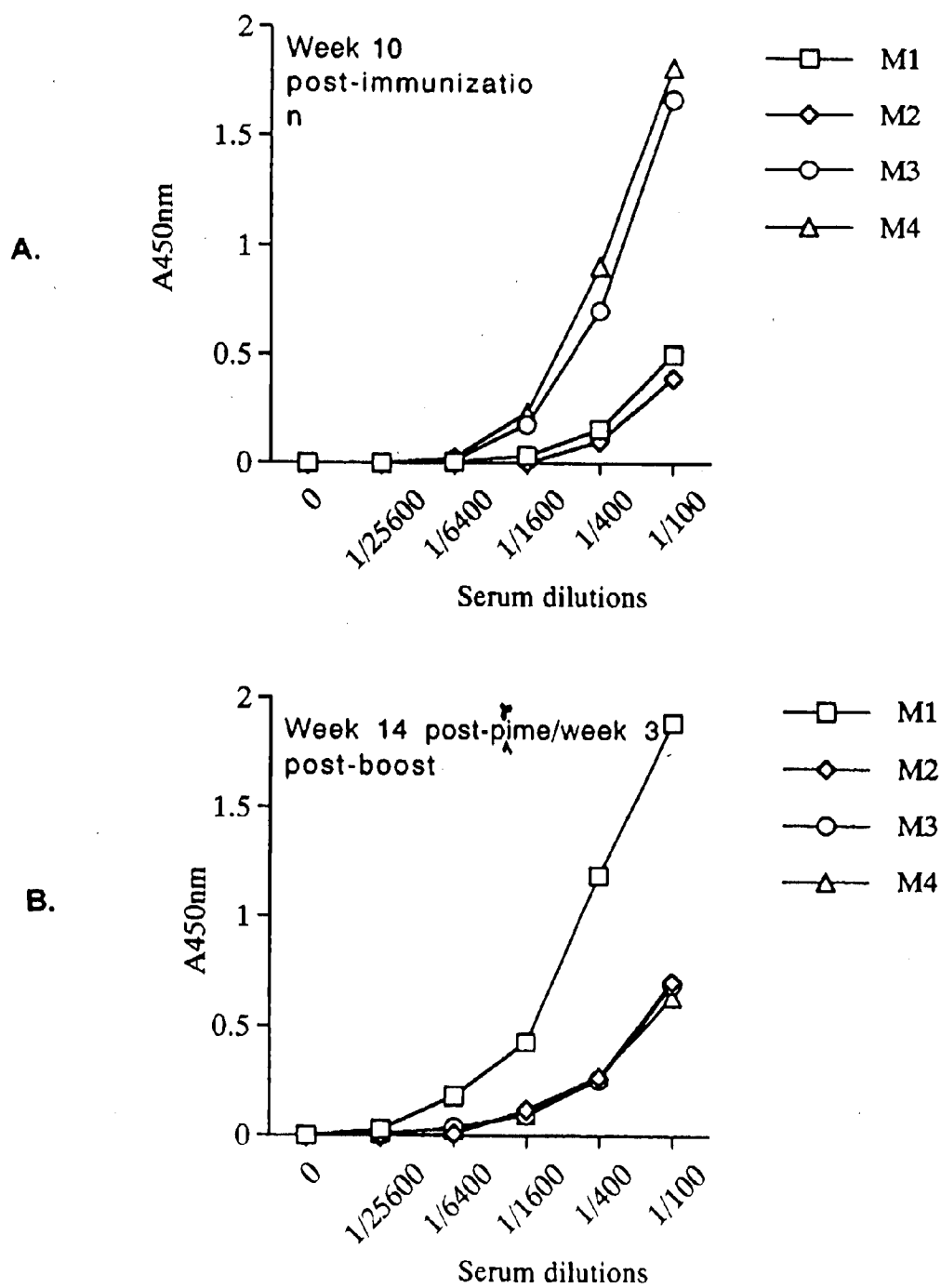
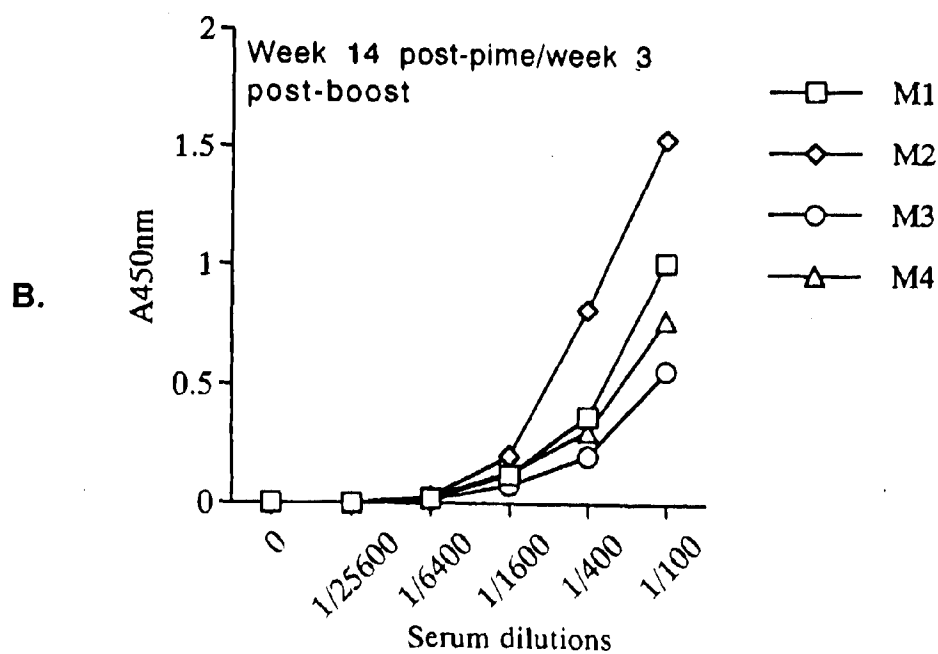
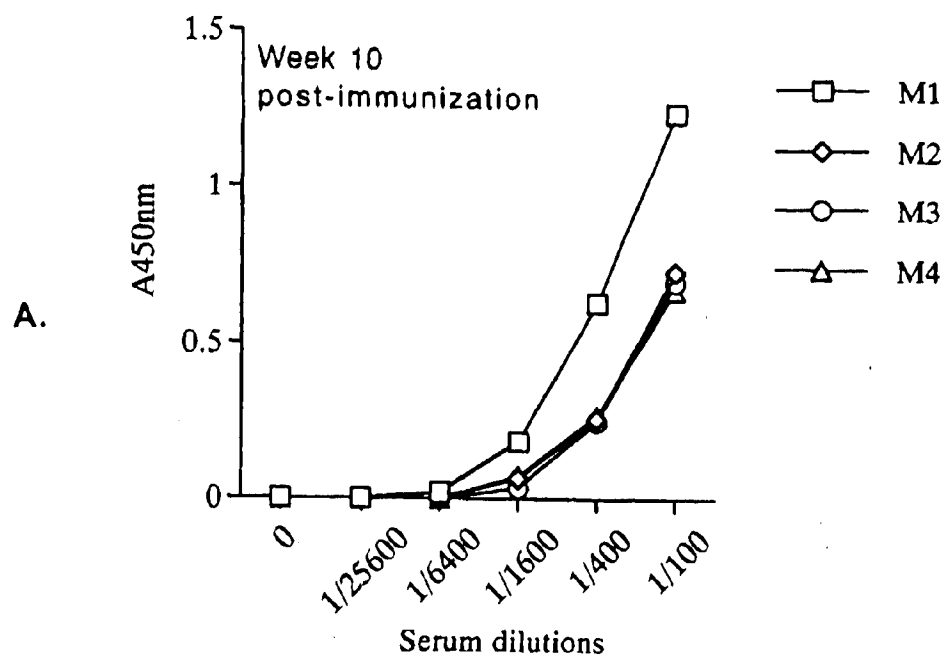
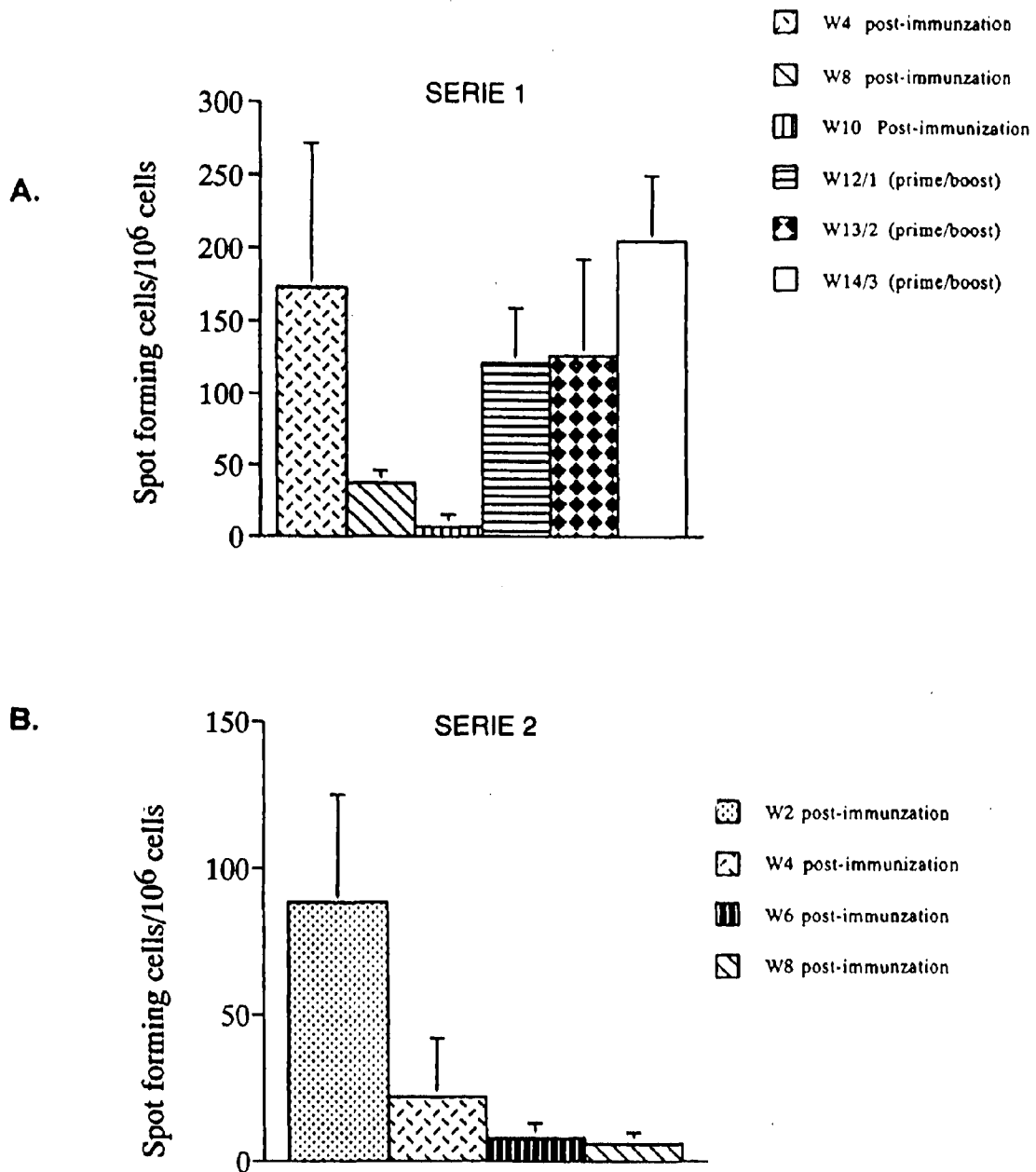


FIGURE 13



**FIGURE 14**

Gag-specific IFN $\gamma$  secreting splenic cells  
after immunization of mice with Ad(3C,  
Gag, Env)



19/97

**FIGURE 15** L23: ELISPOT for IFN $\gamma$  secretion: Serie1 spleen cells from mice at week W13/2 (post-prime/boost)

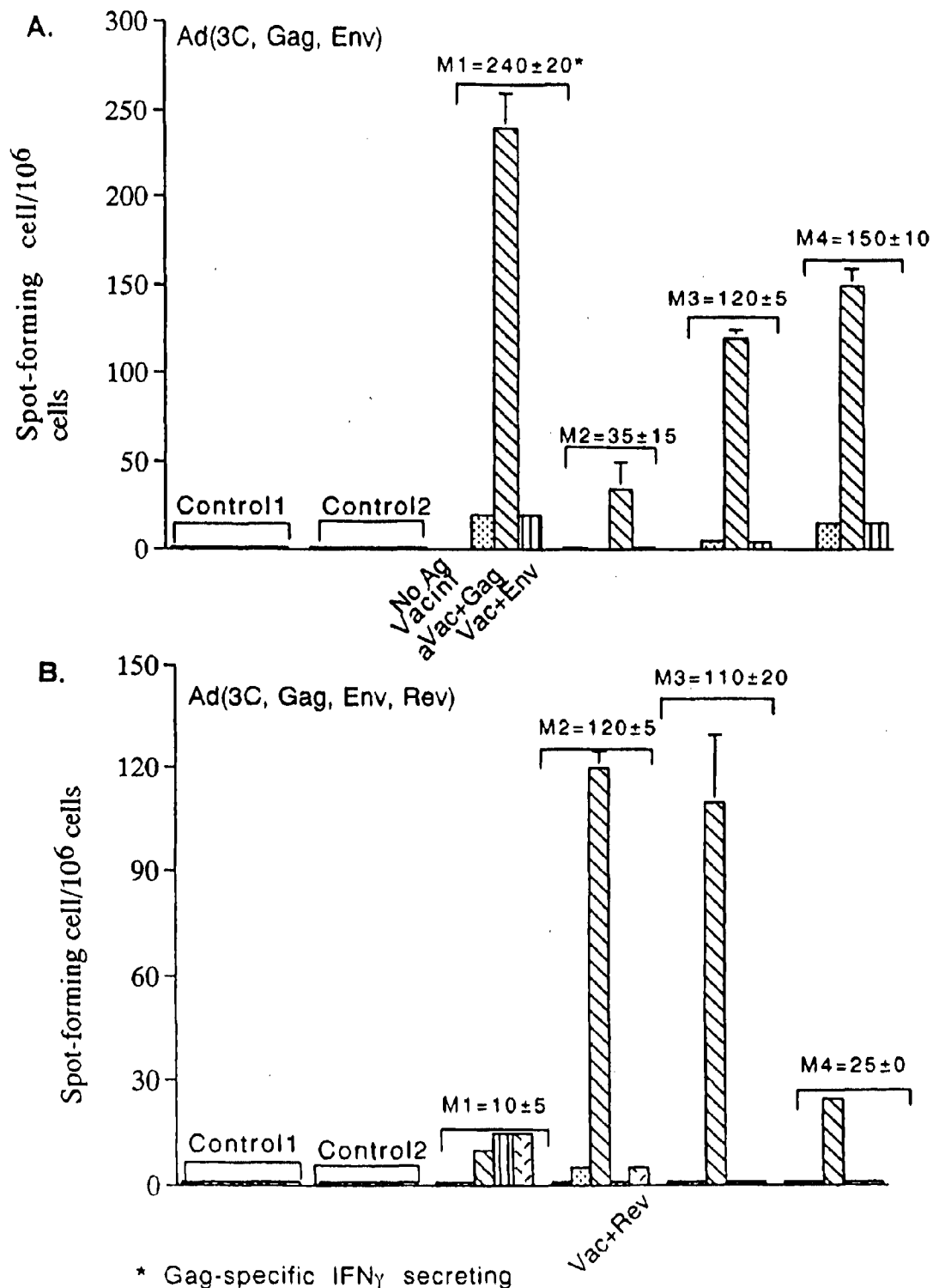


FIGURE 16A

pLAd-E.T.R

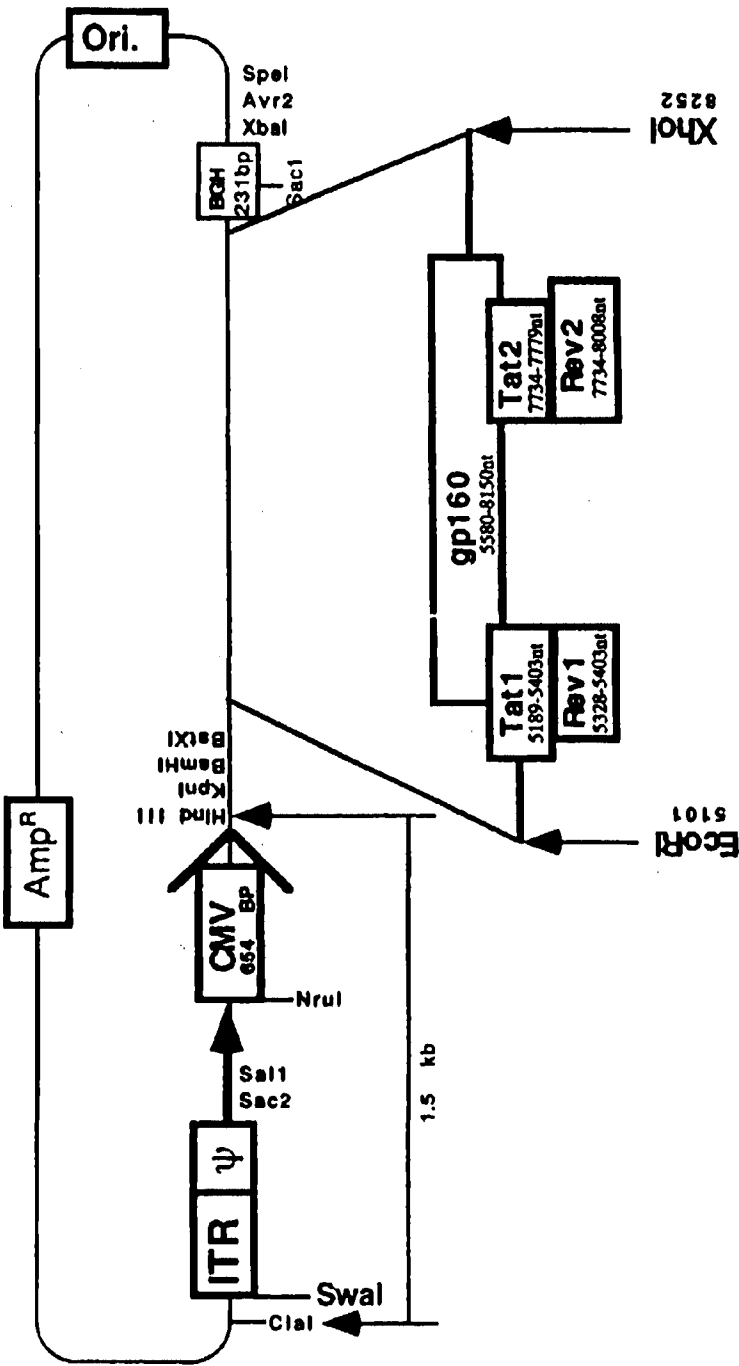


FIGURE 16B

pRad.ORF6-IL2

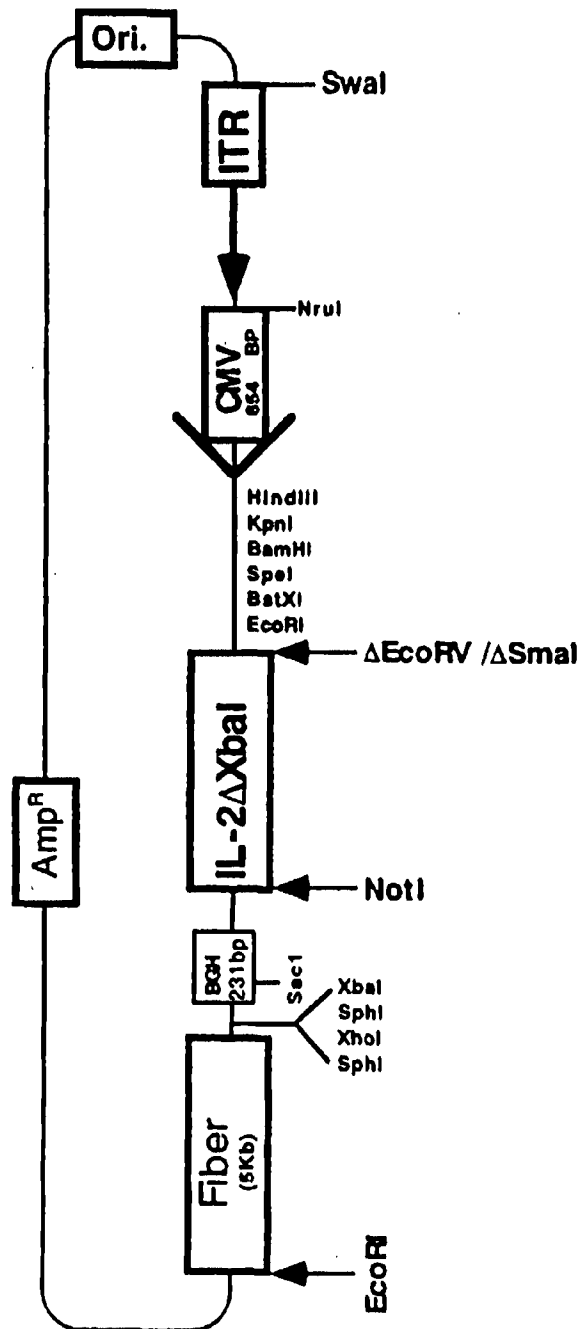


FIGURE 17A

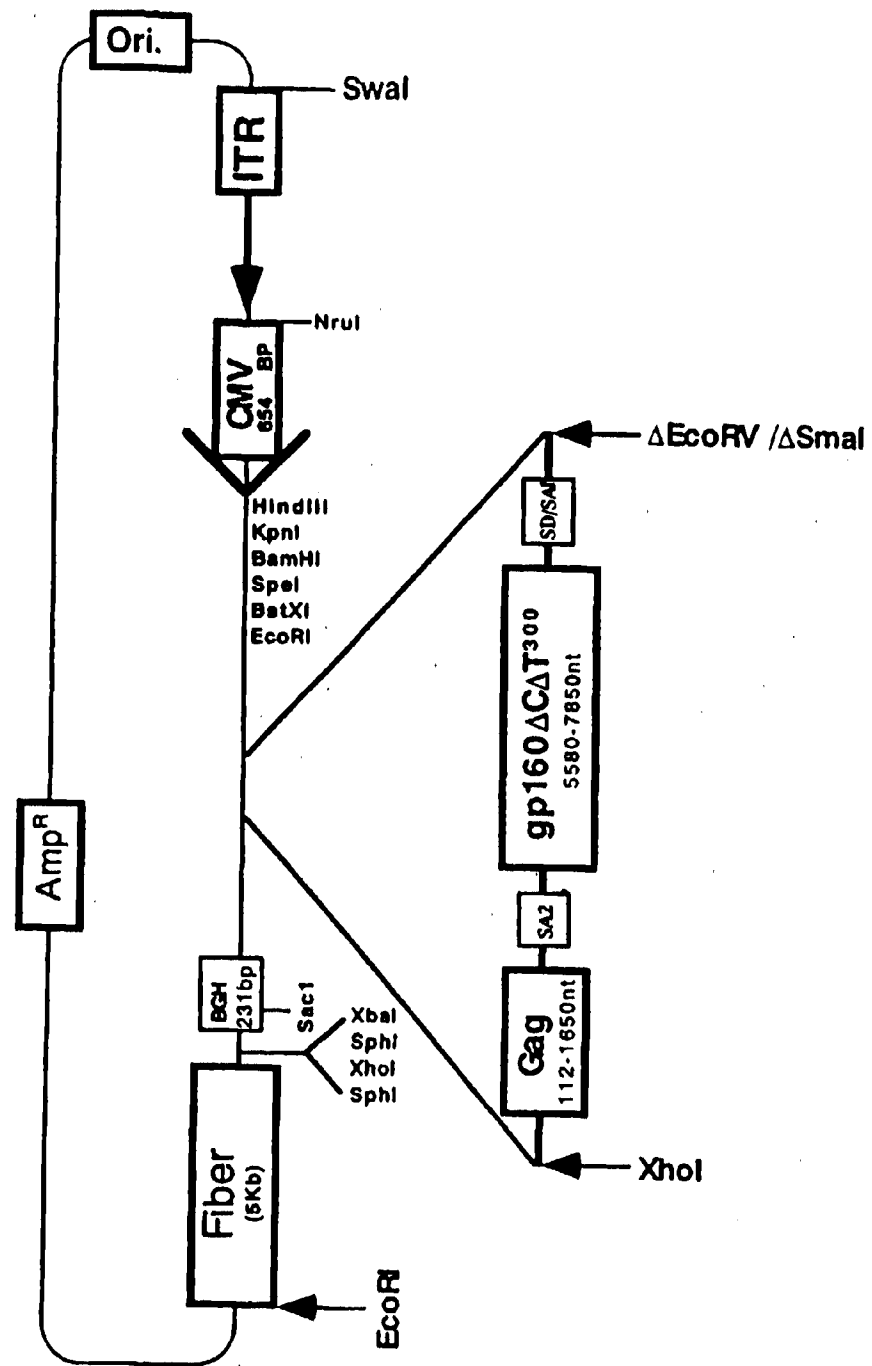
pRad.ORF6-E<sup>m</sup>ΔCΔT<sup>300</sup>-G

FIGURE 17B

pLAd-3C

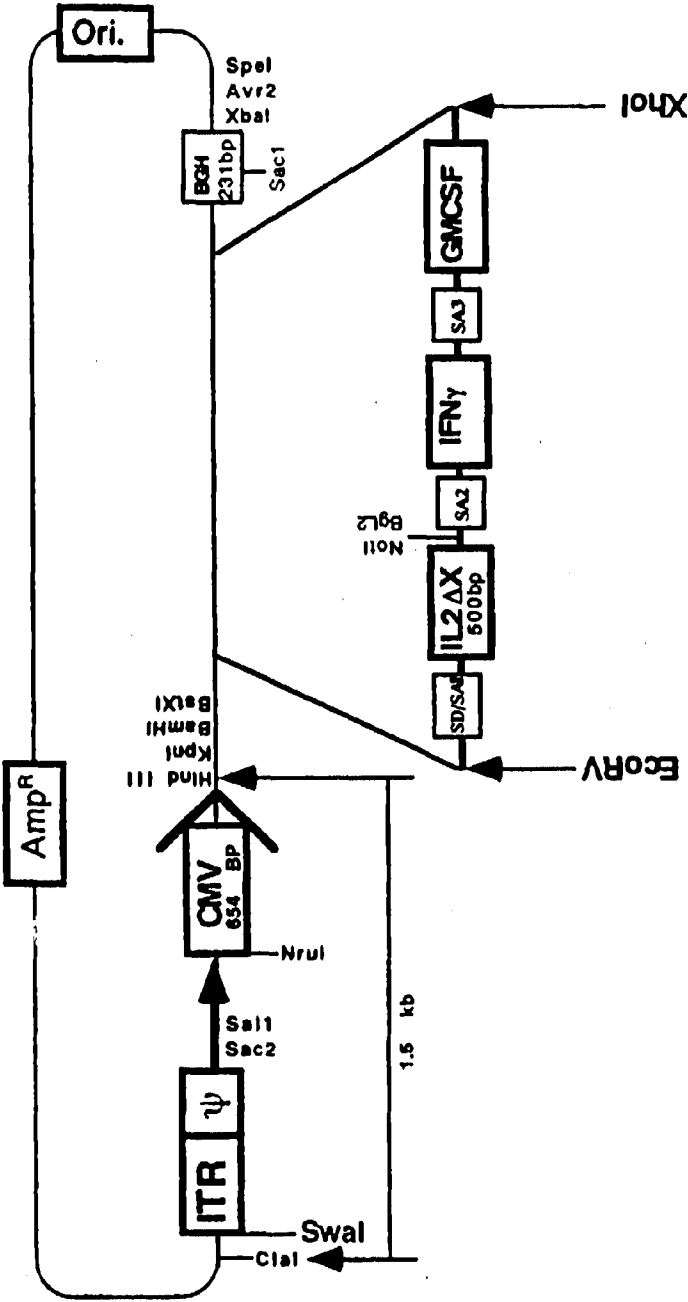




FIGURE 18

pRAD.ORF6-E<sup>Δ</sup>ΔCAT<sup>99</sup>.T.R-G

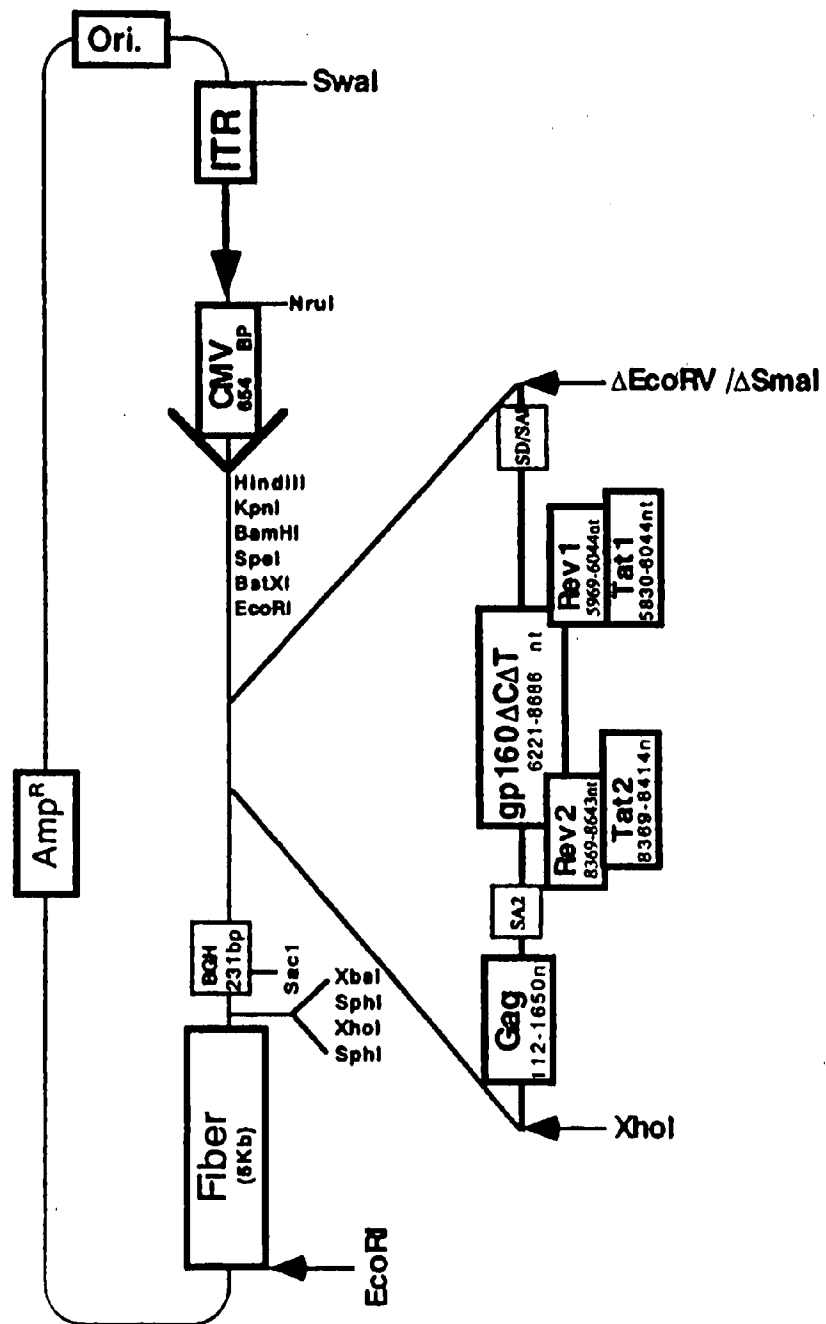


FIGURE 19A  
pLAd-E<sup>m</sup>ΔV<sub>1,2</sub>ΔCAT.T.R-IL2

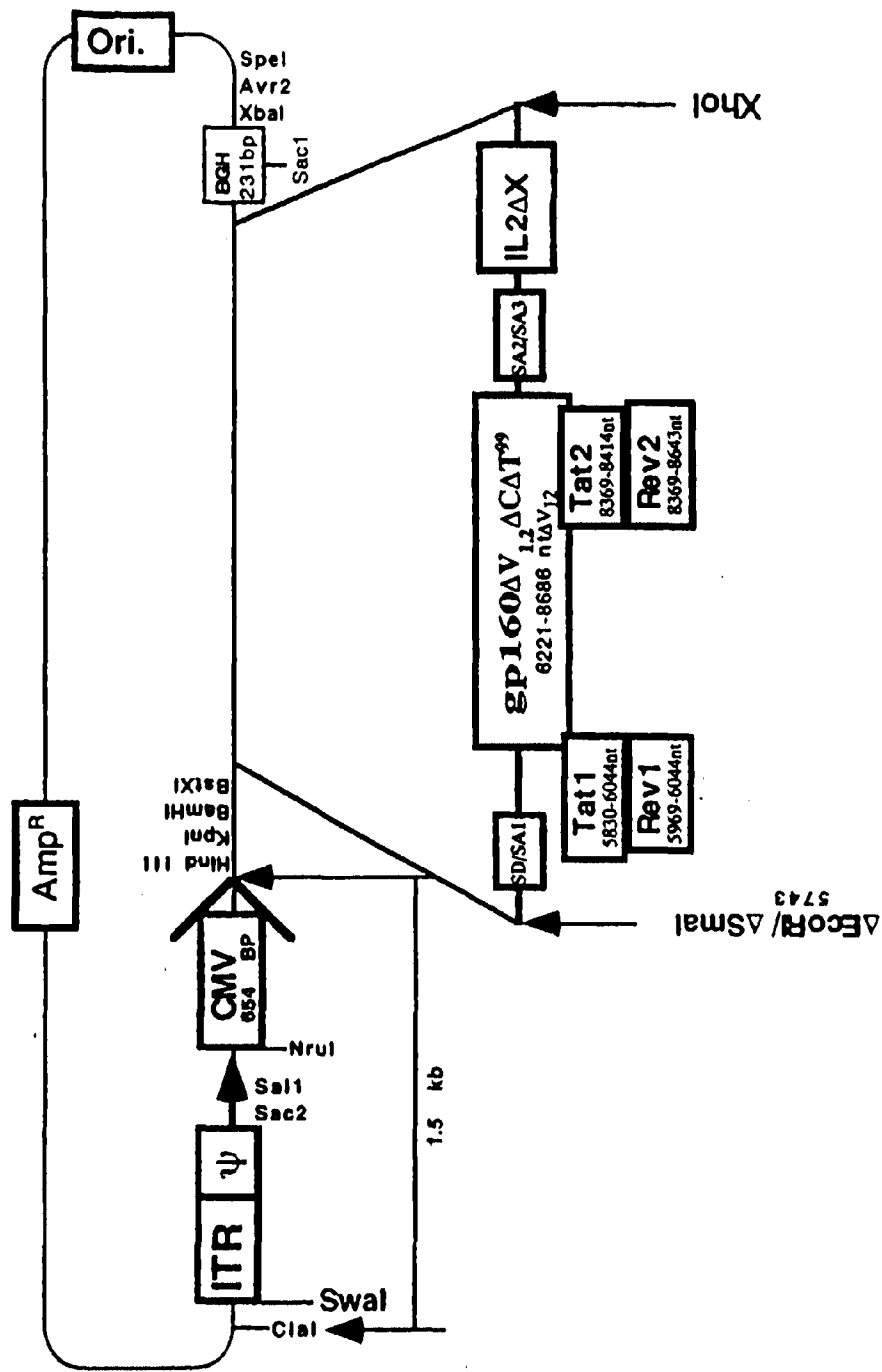


FIGURE 19B

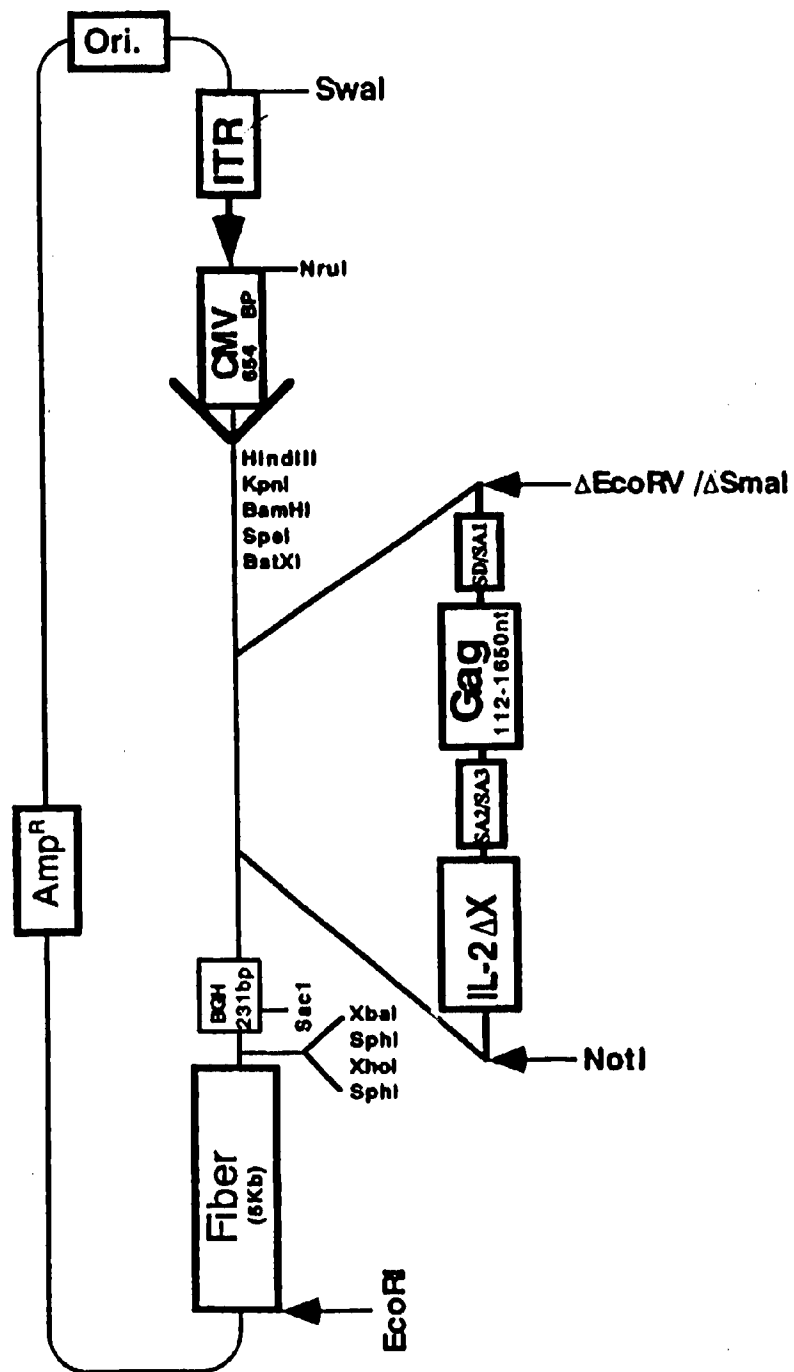
pRA<sub>d</sub>.ORF6-G.IL2

FIGURE 20

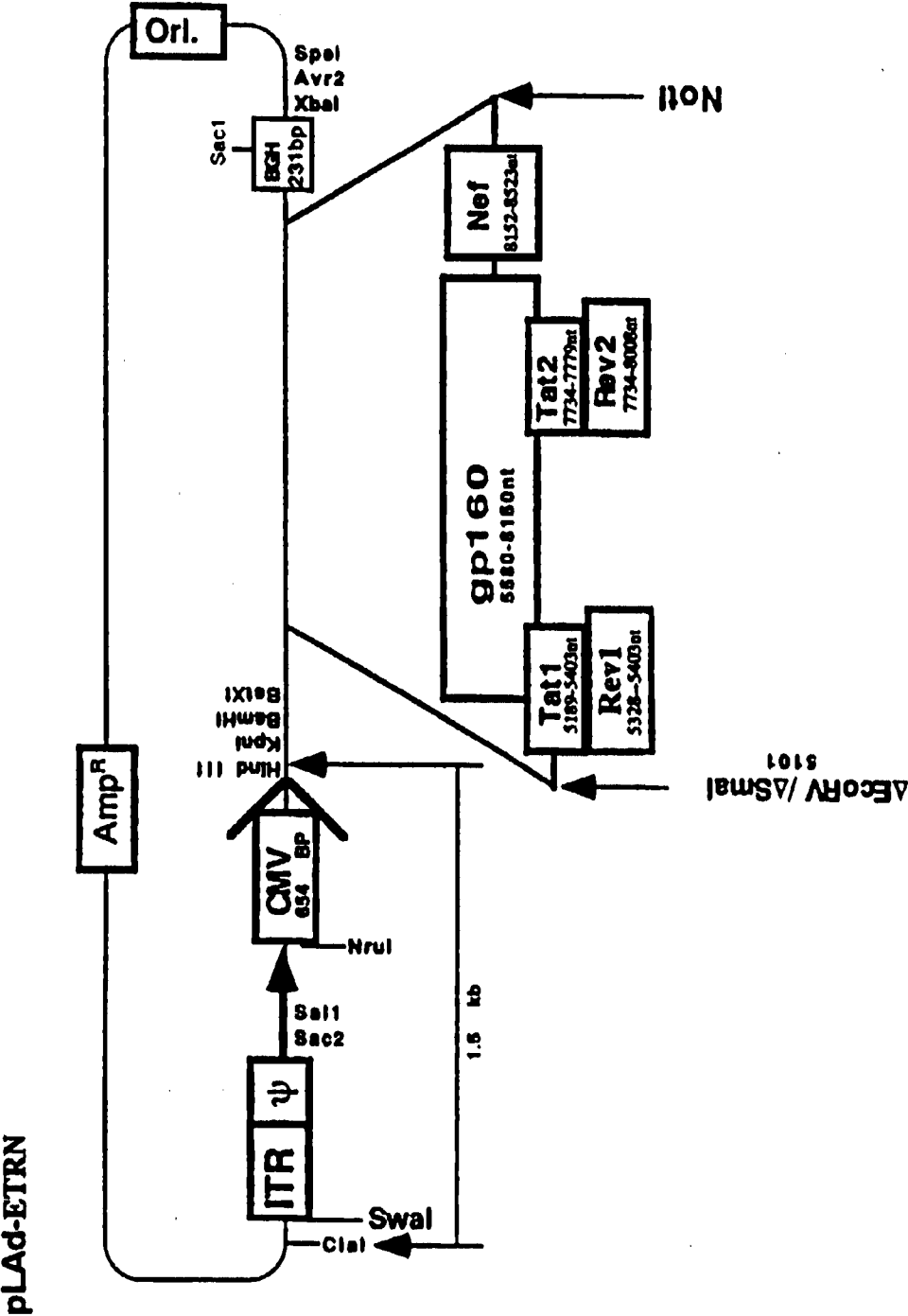


FIGURE 21  
pLAd-E<sup>m</sup>ΔC.N

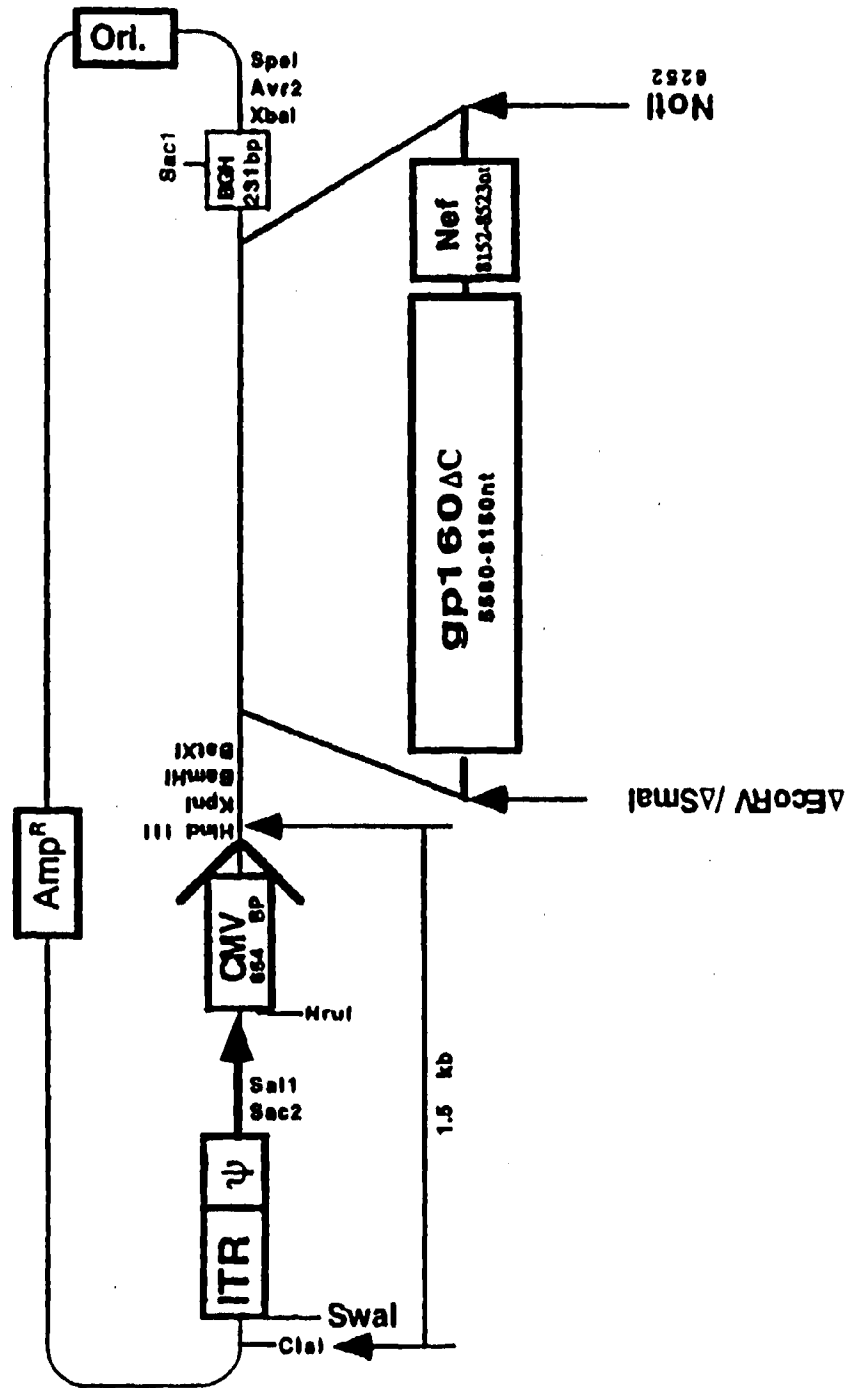
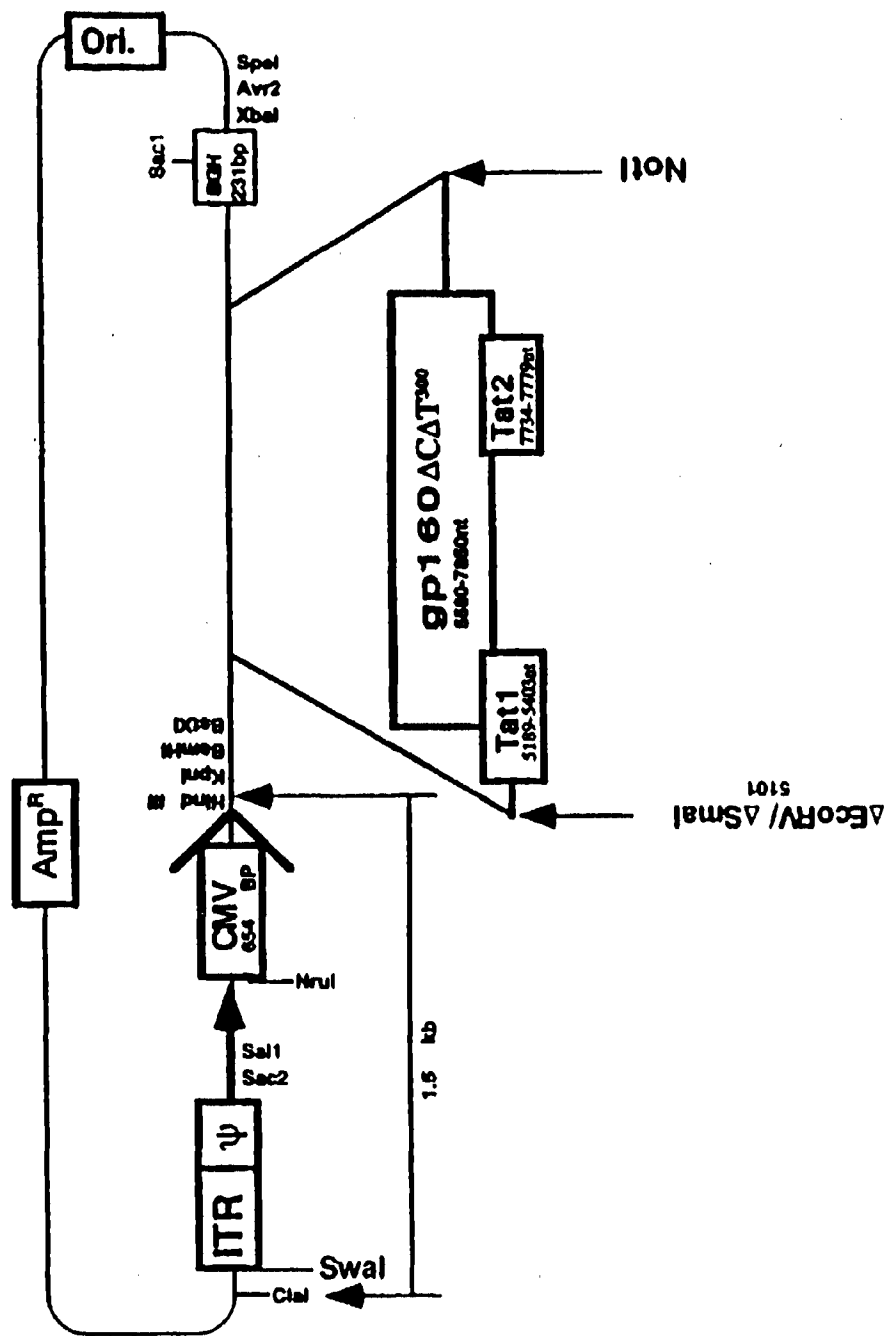
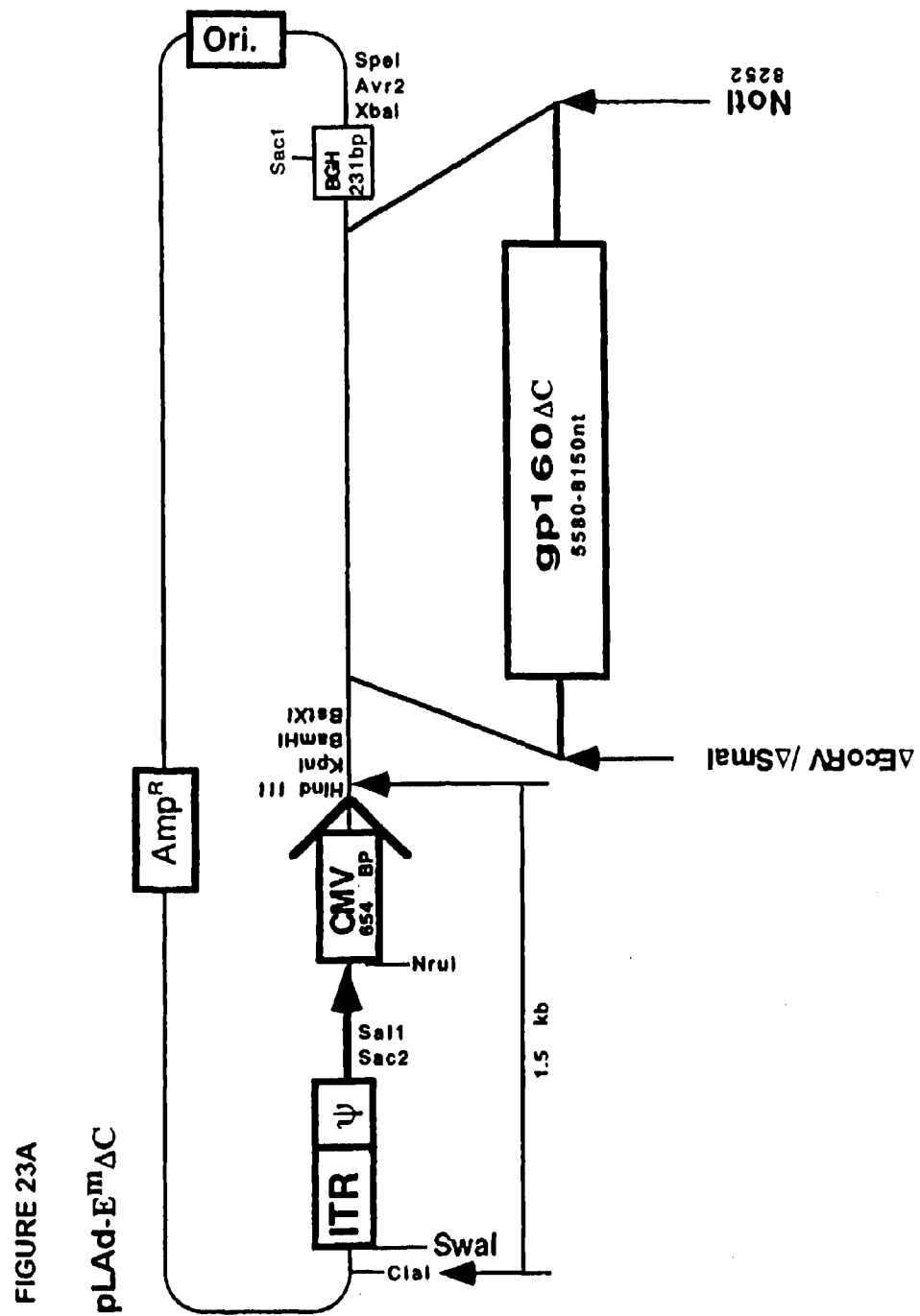


FIGURE 22

pLAd-E<sup>+</sup>ΔCAT<sup>300</sup>.T





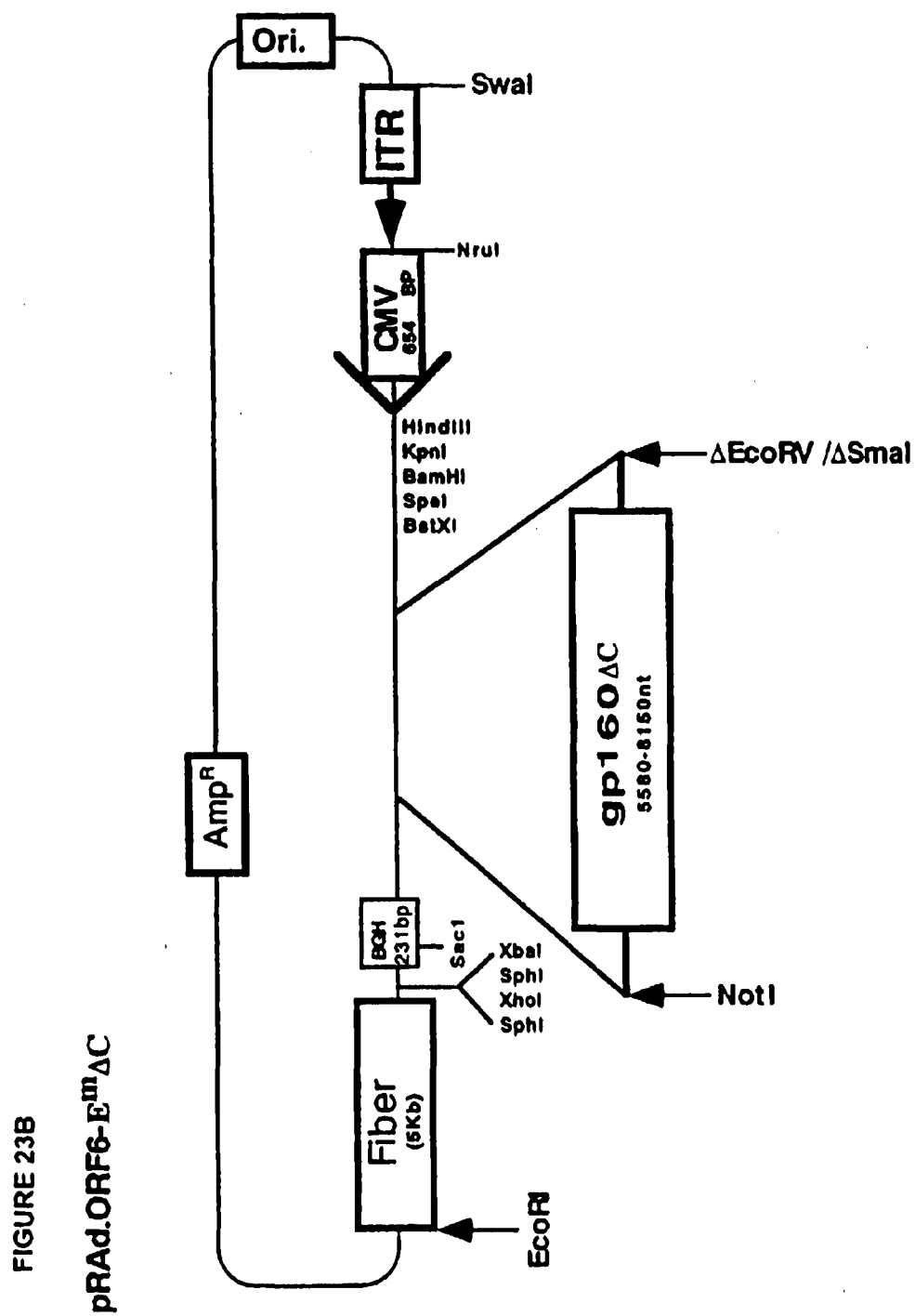
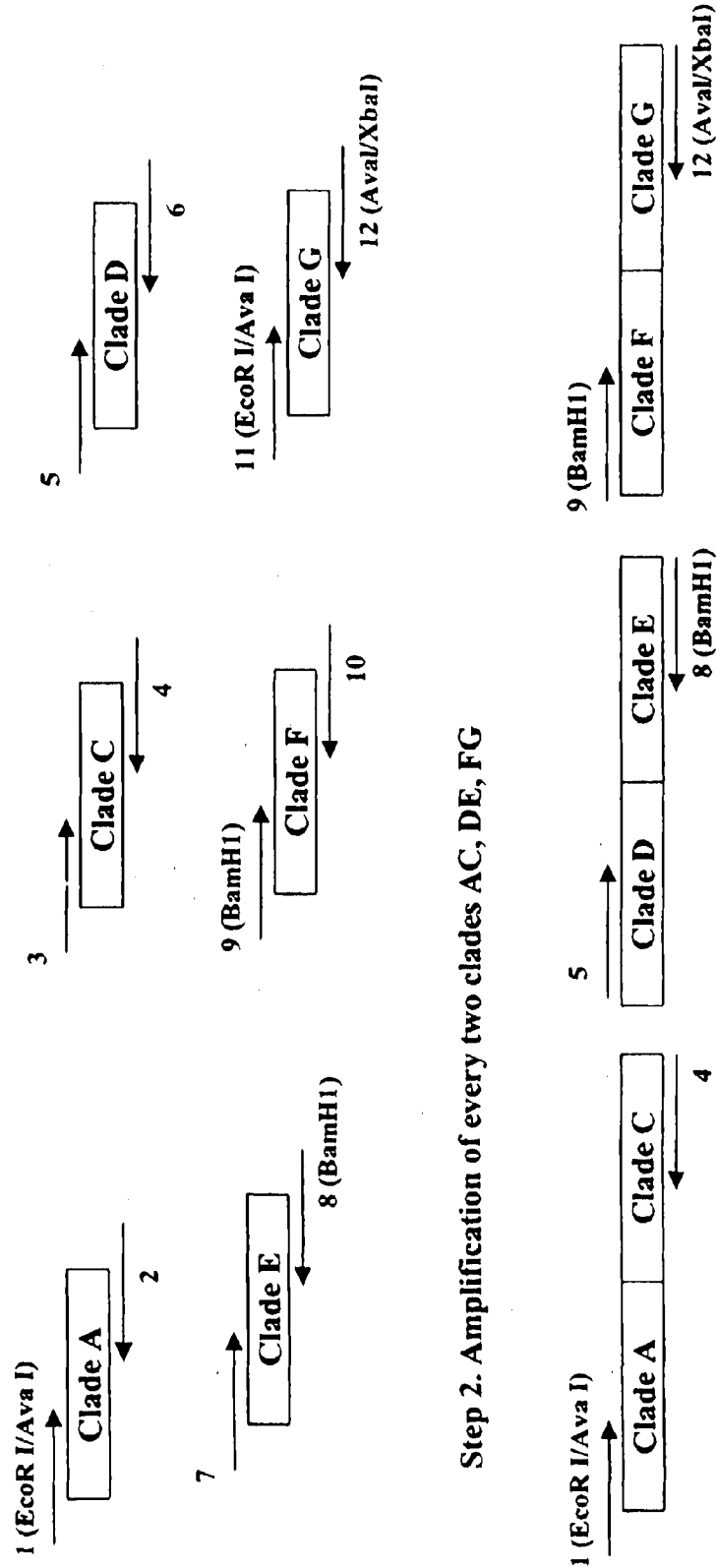


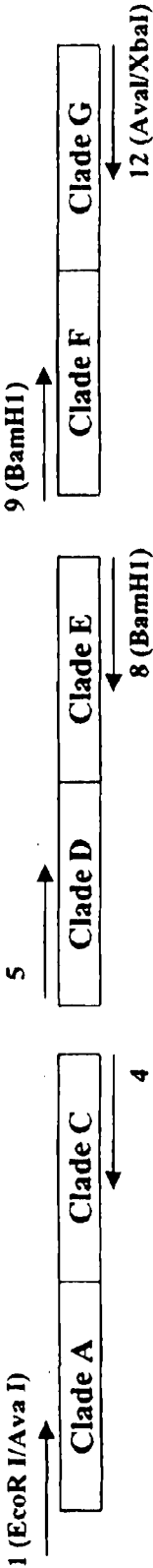


FIGURE 24

Step 1. Amplification of each individual clade A-G

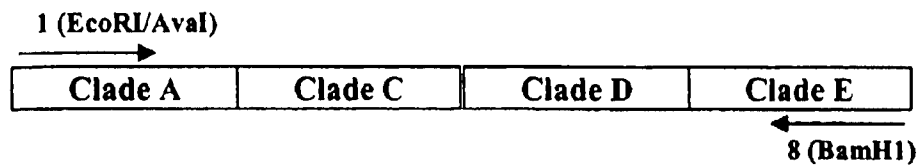


Step 2. Amplification of every two clades AC, DE, FG

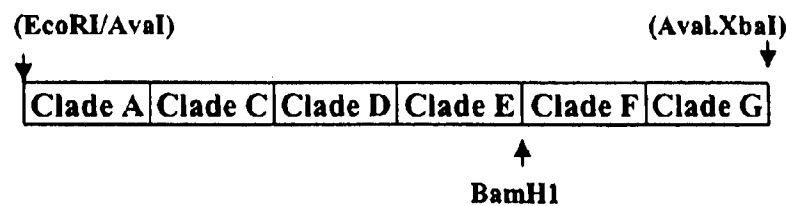


**FIGURE 24-cont.**

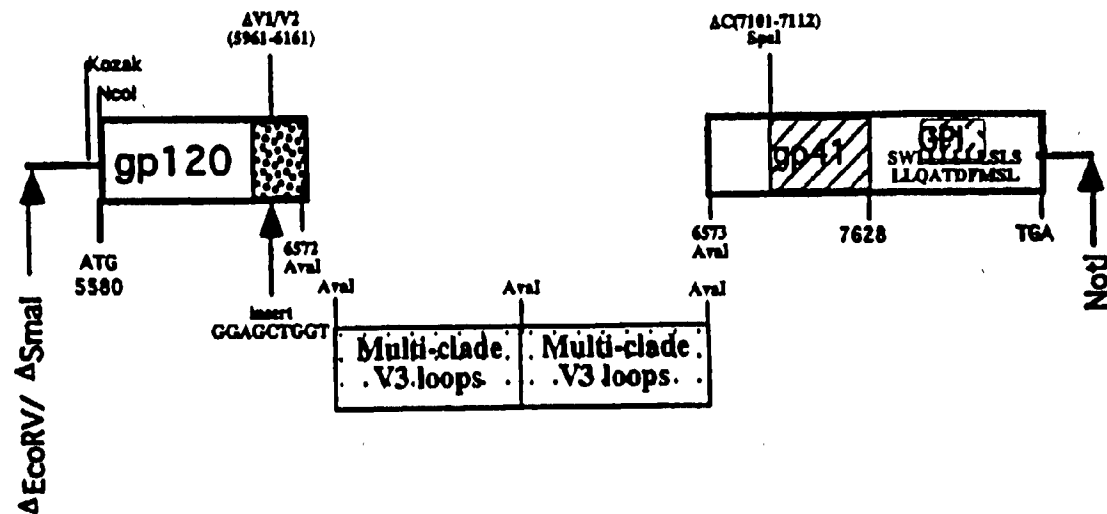
### Step 3. Amplification of clades ACDE



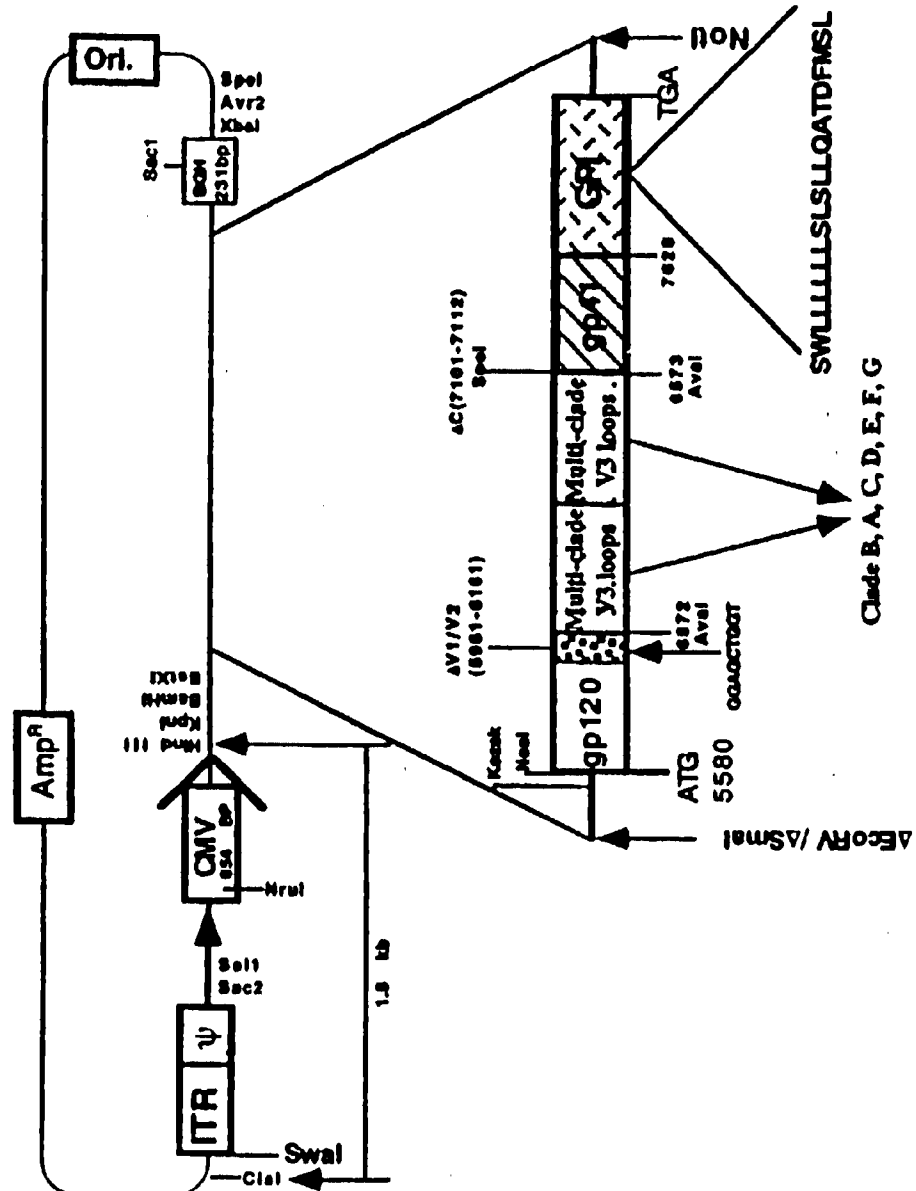
#### Step 4. Cloning the multi-clades into pSP73 vector



### Step 5. Generation of duplicated multi-clades







**FIGURE 26**

FIGURE 27A

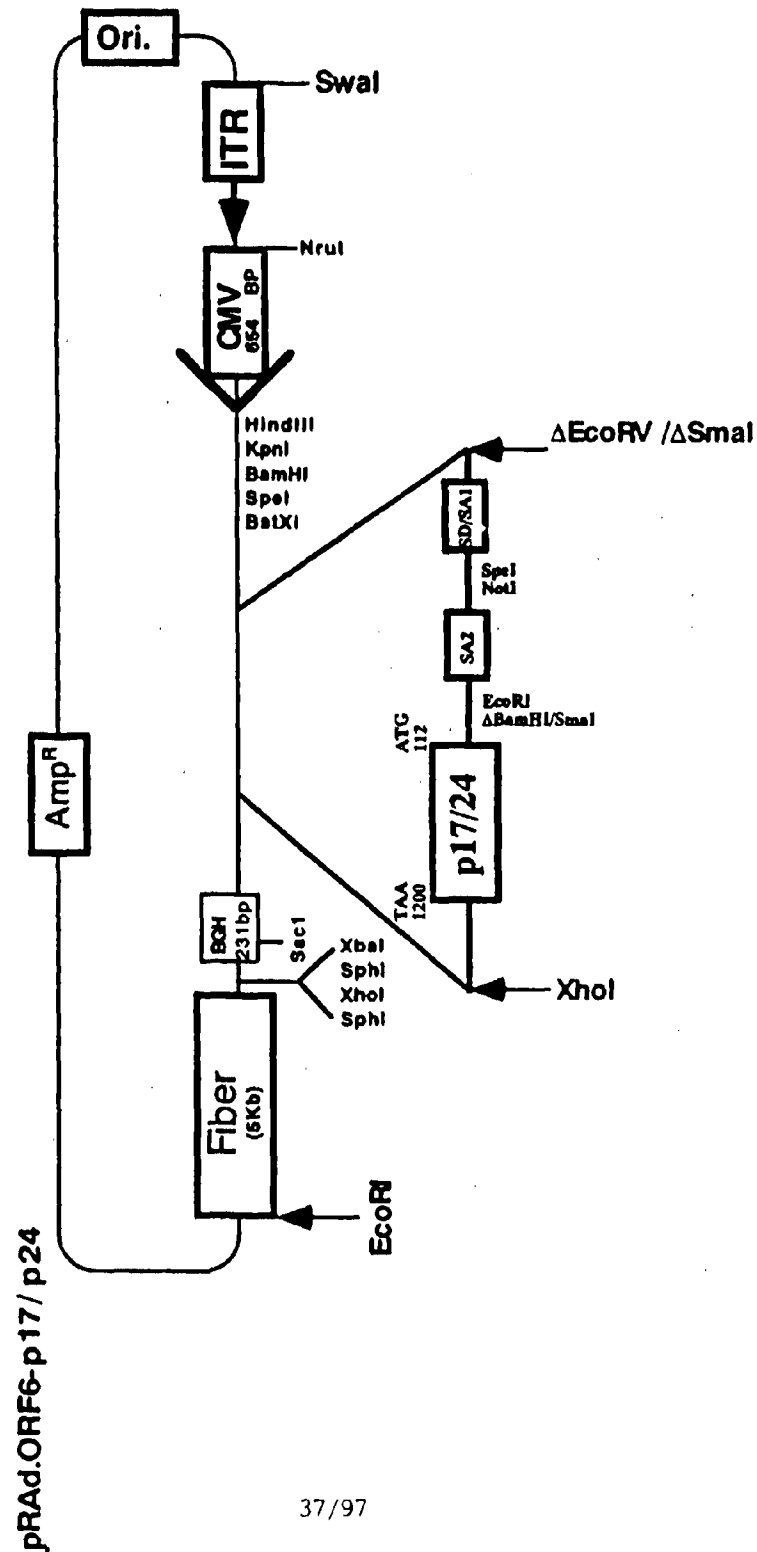
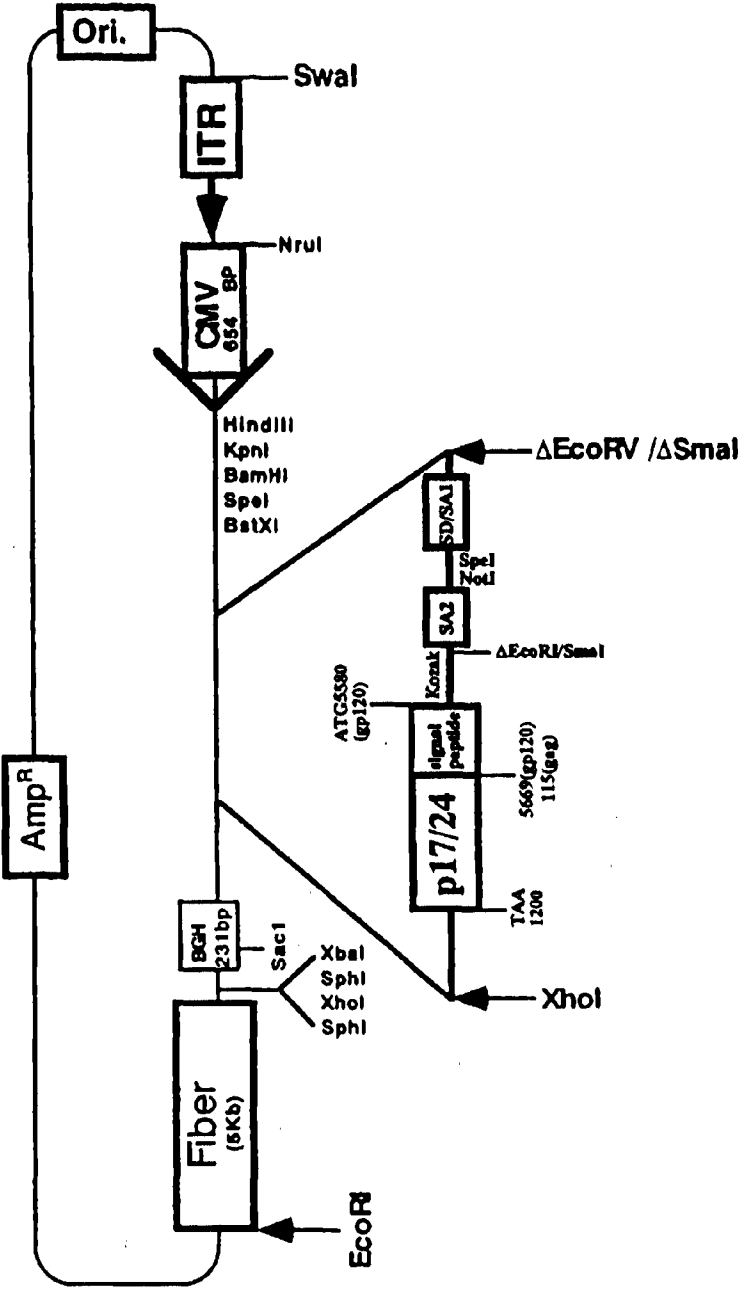


FIGURE 27B

pRad. ORF6-p17/24sec



**FIGURE 27C**

**RAAd.ORF6-p17/24MB**

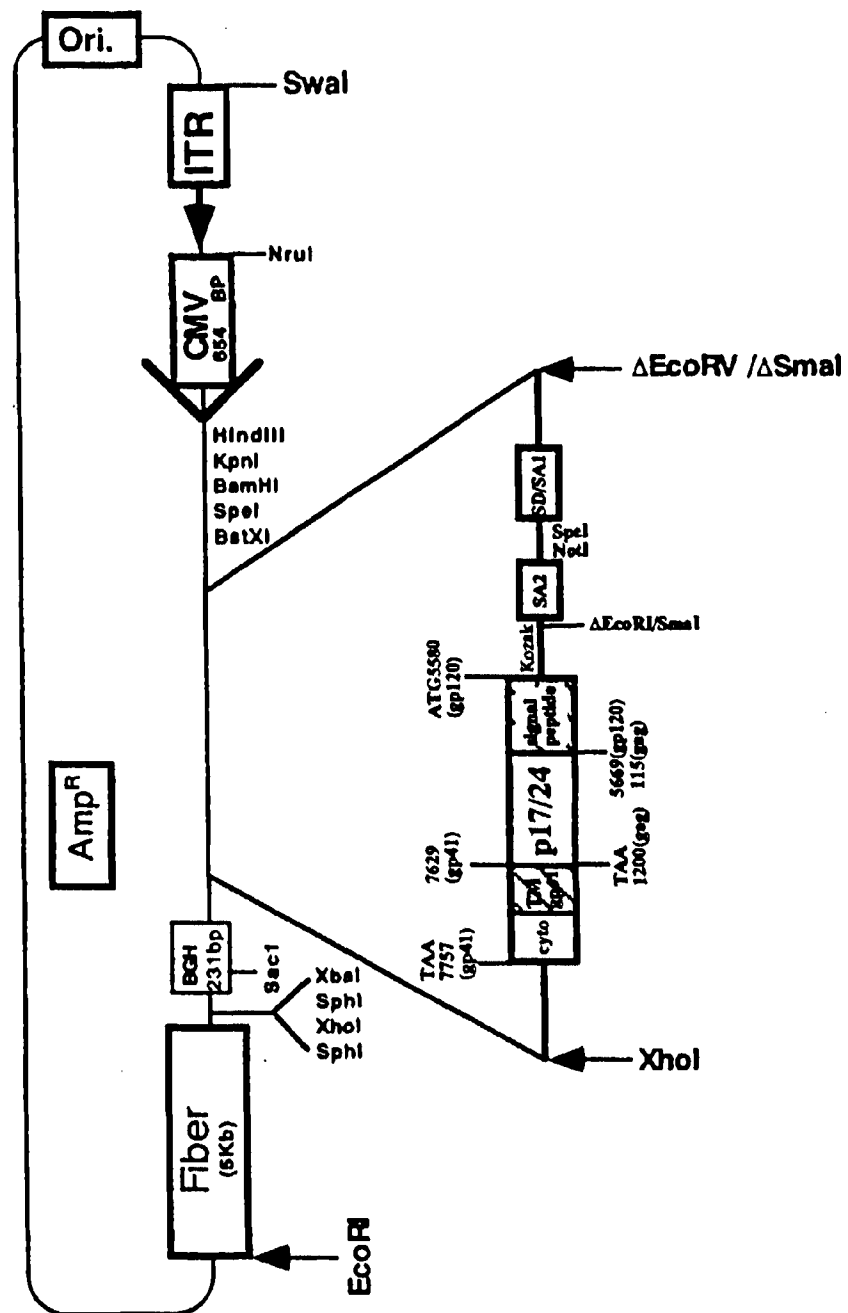


FIGURE 28A

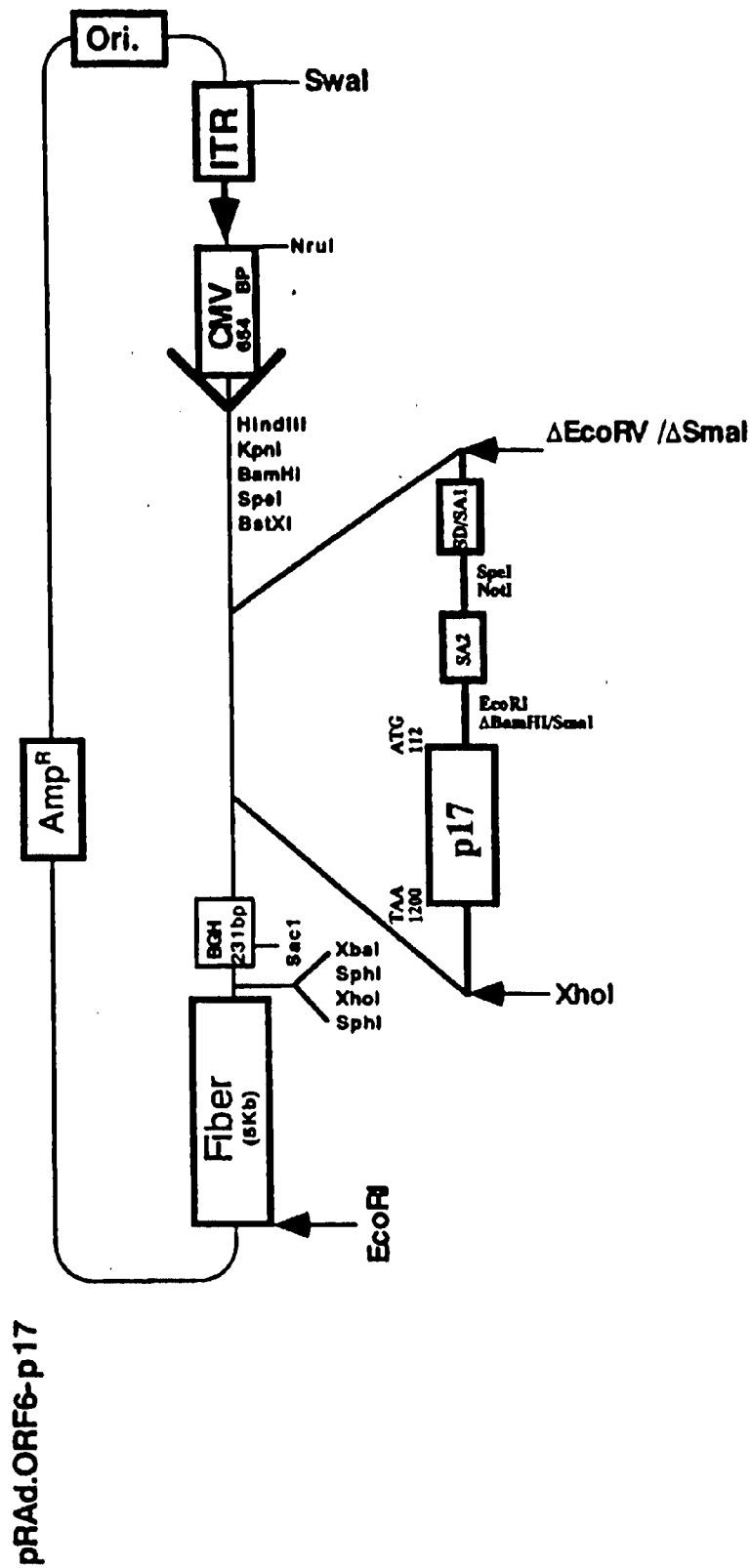




FIGURE 28B

pRAD. ORF6-p17 sec

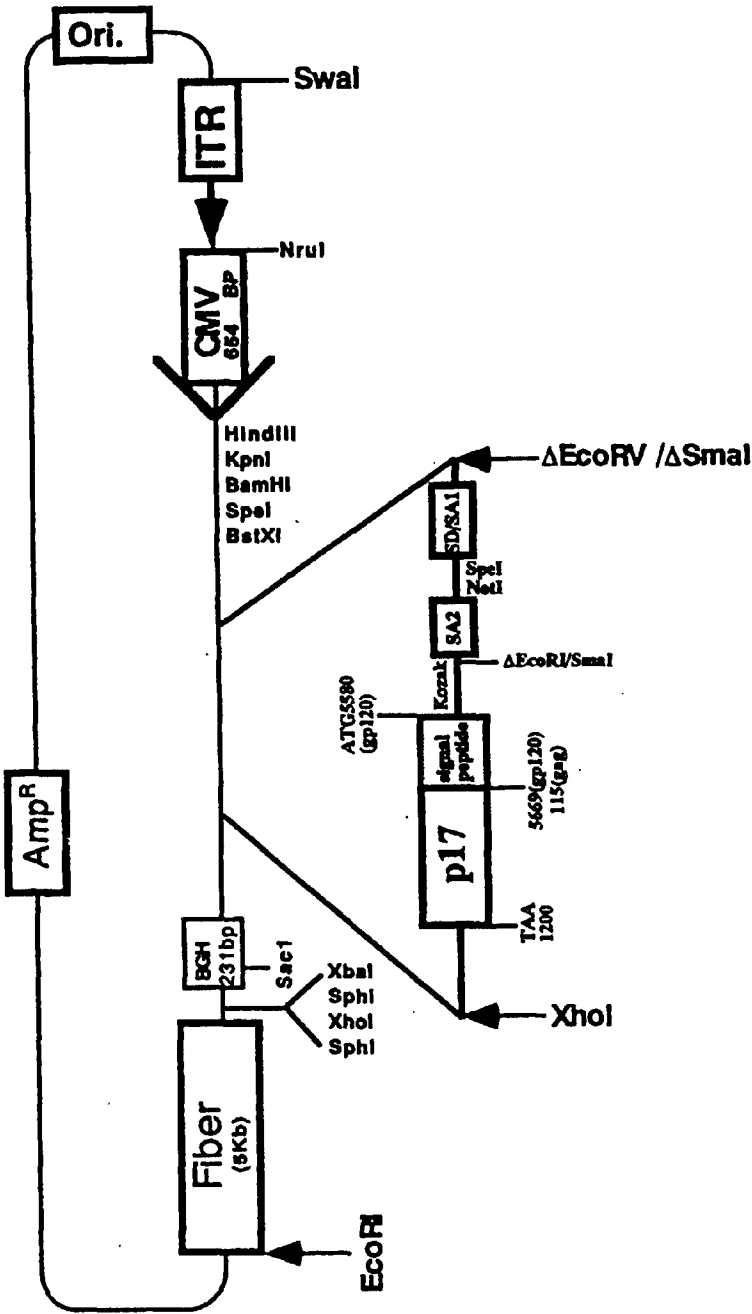




FIGURE 29A

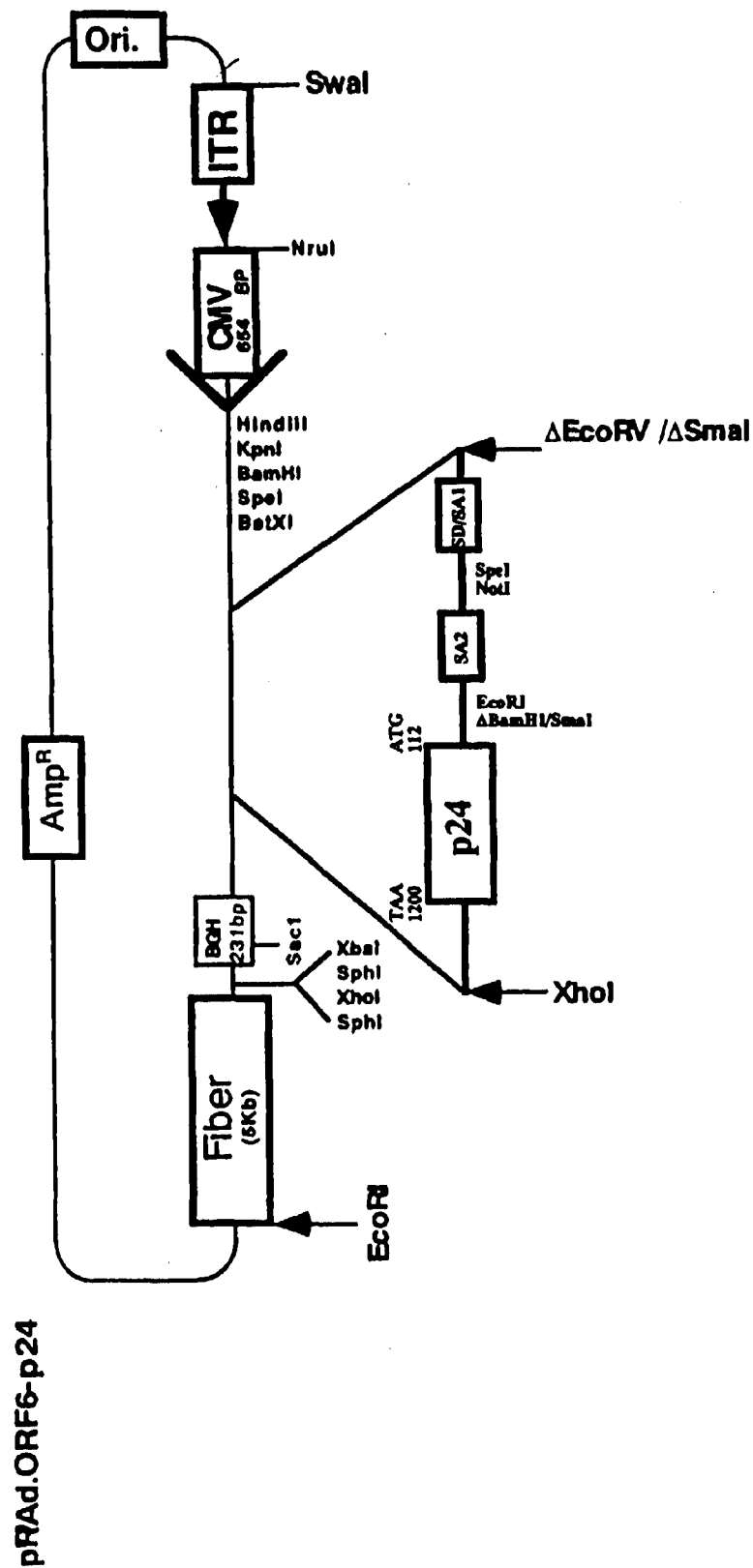


FIGURE 29B

pRad.ORIF6- p24 sec

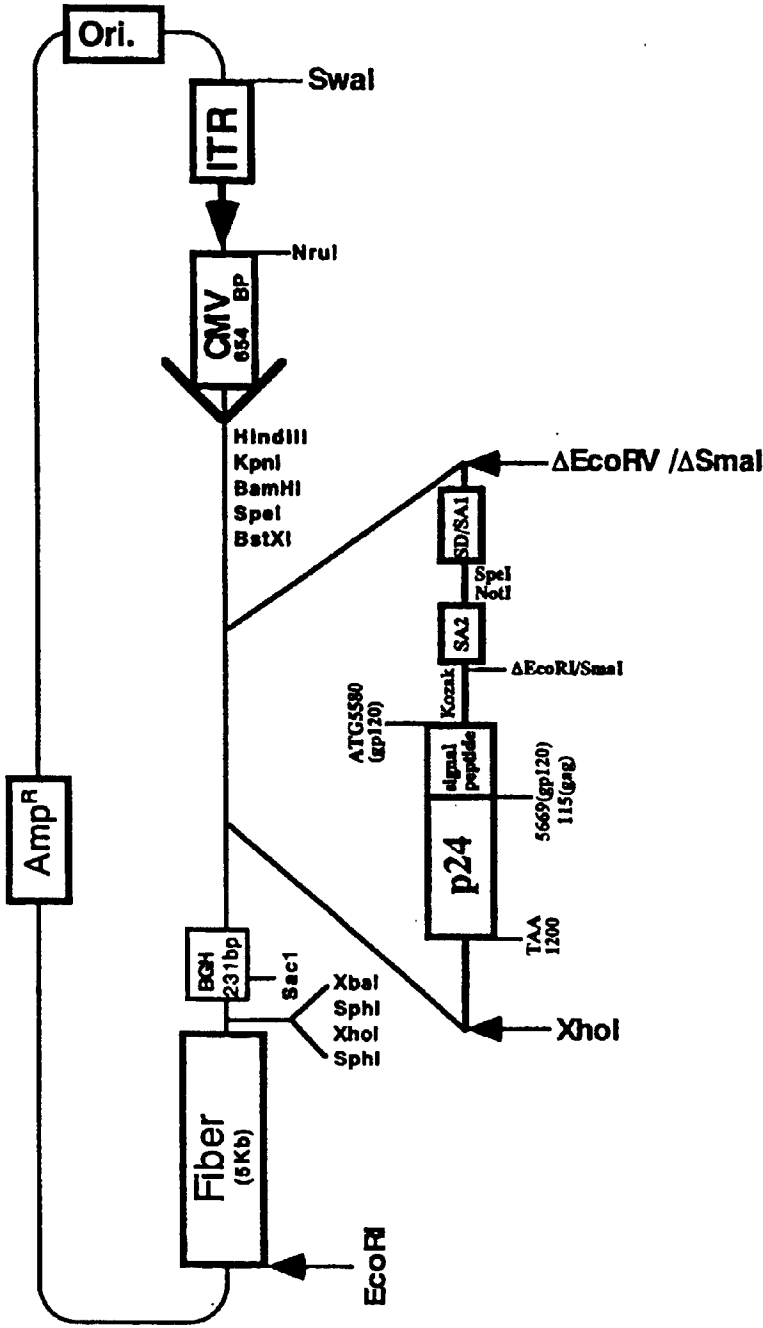


FIGURE 29C

pRAD. ORF6-p24MB

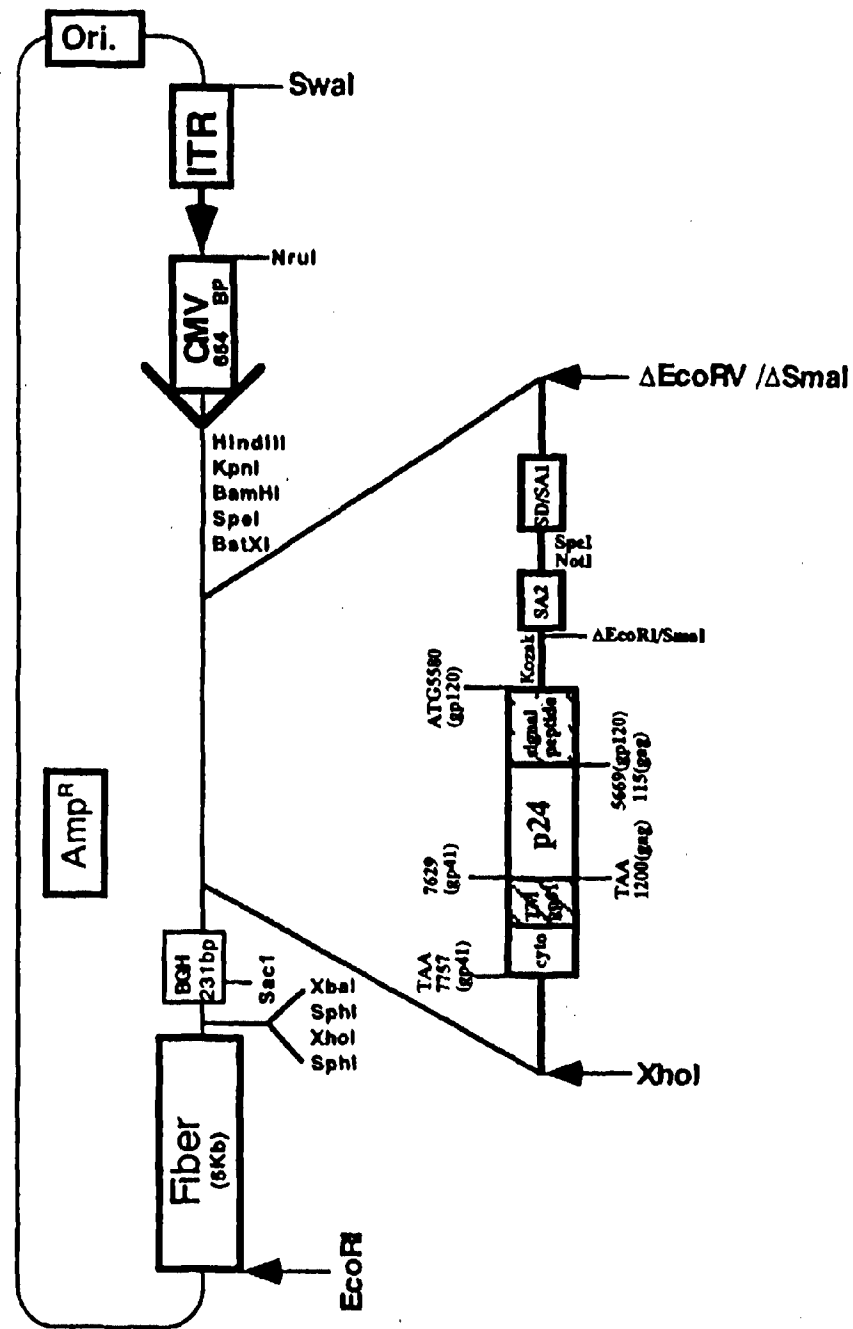


FIGURE 30A

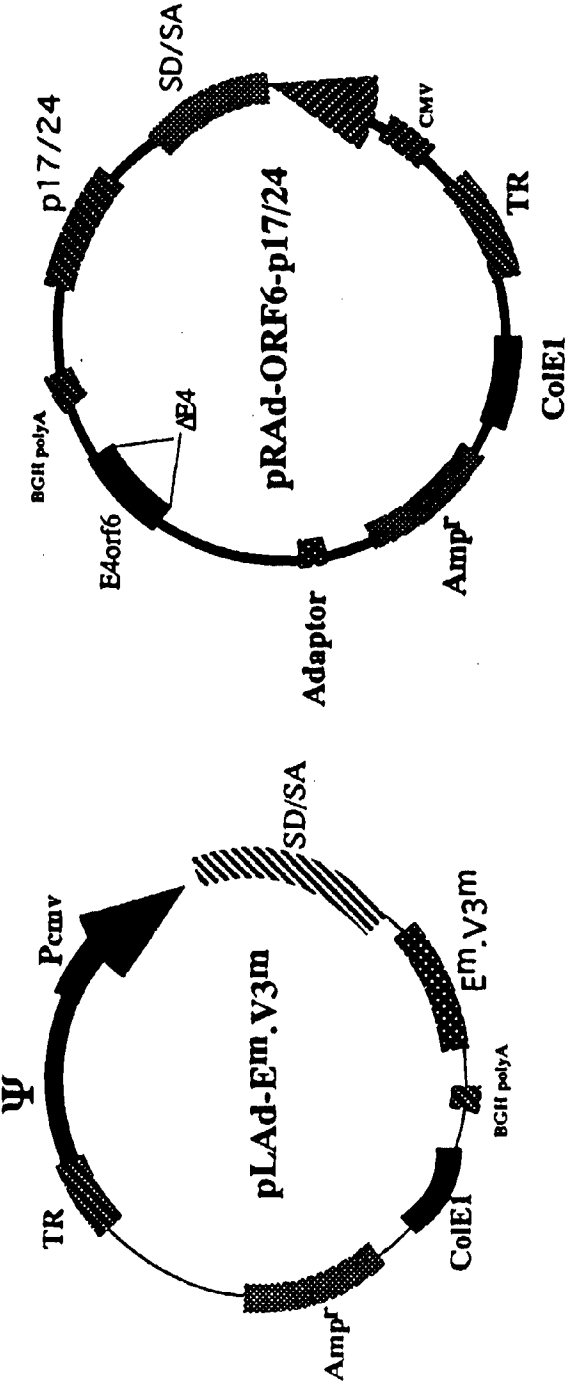
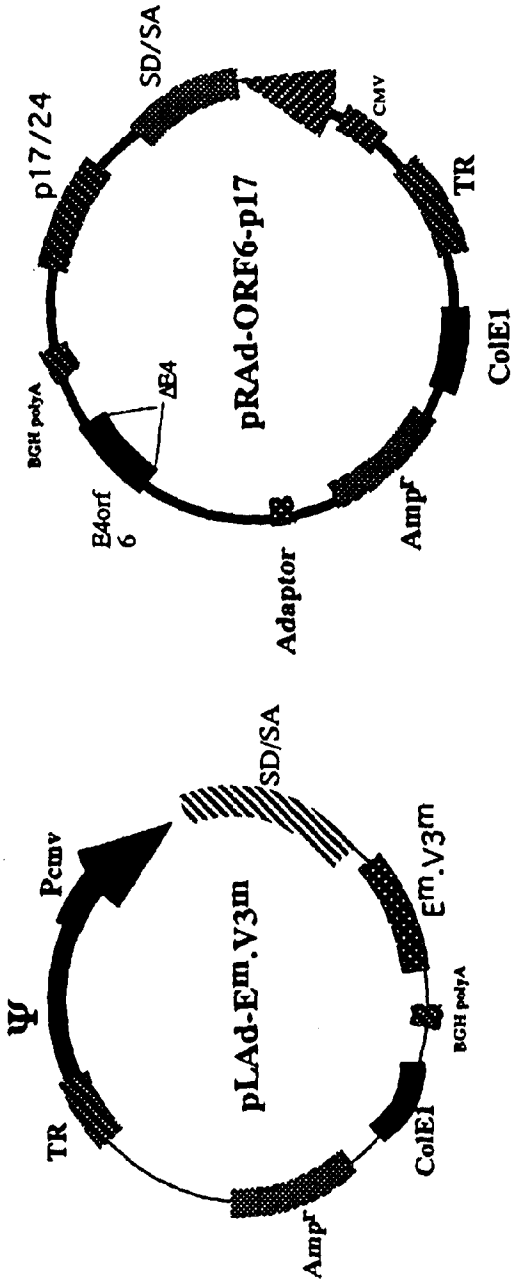




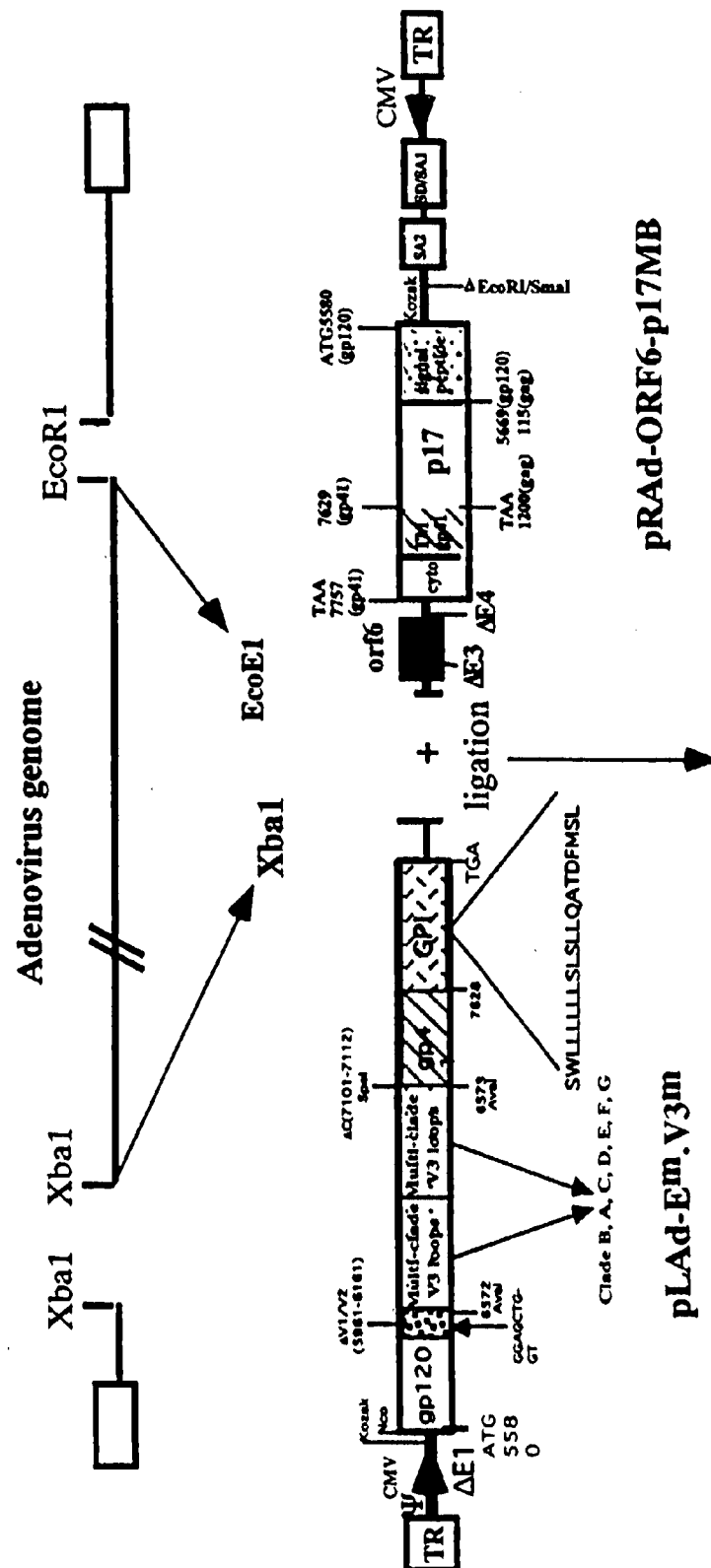




FIGURE 31A



**FIGURE 31B**



**FIGURE 31B-continued**

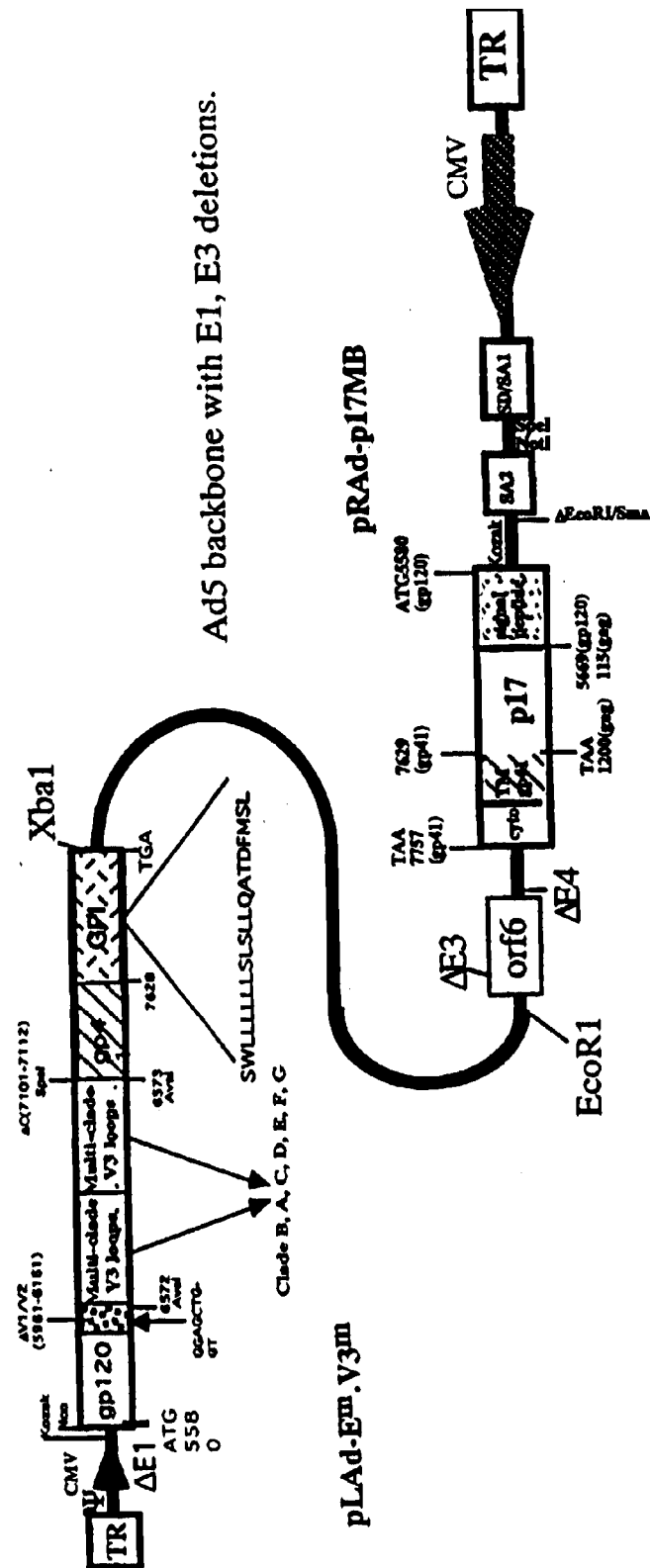
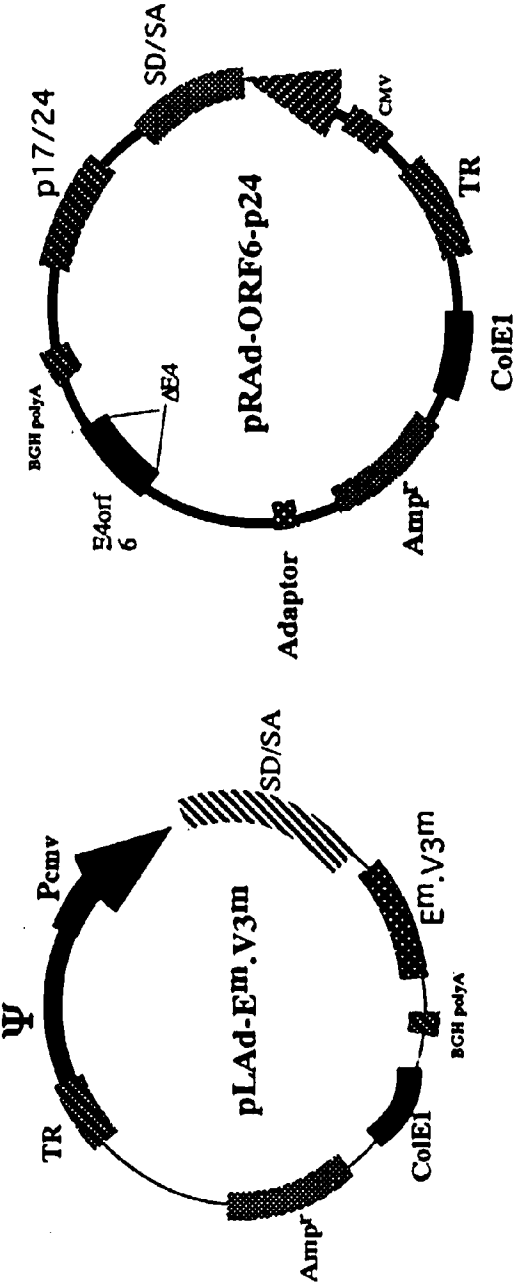
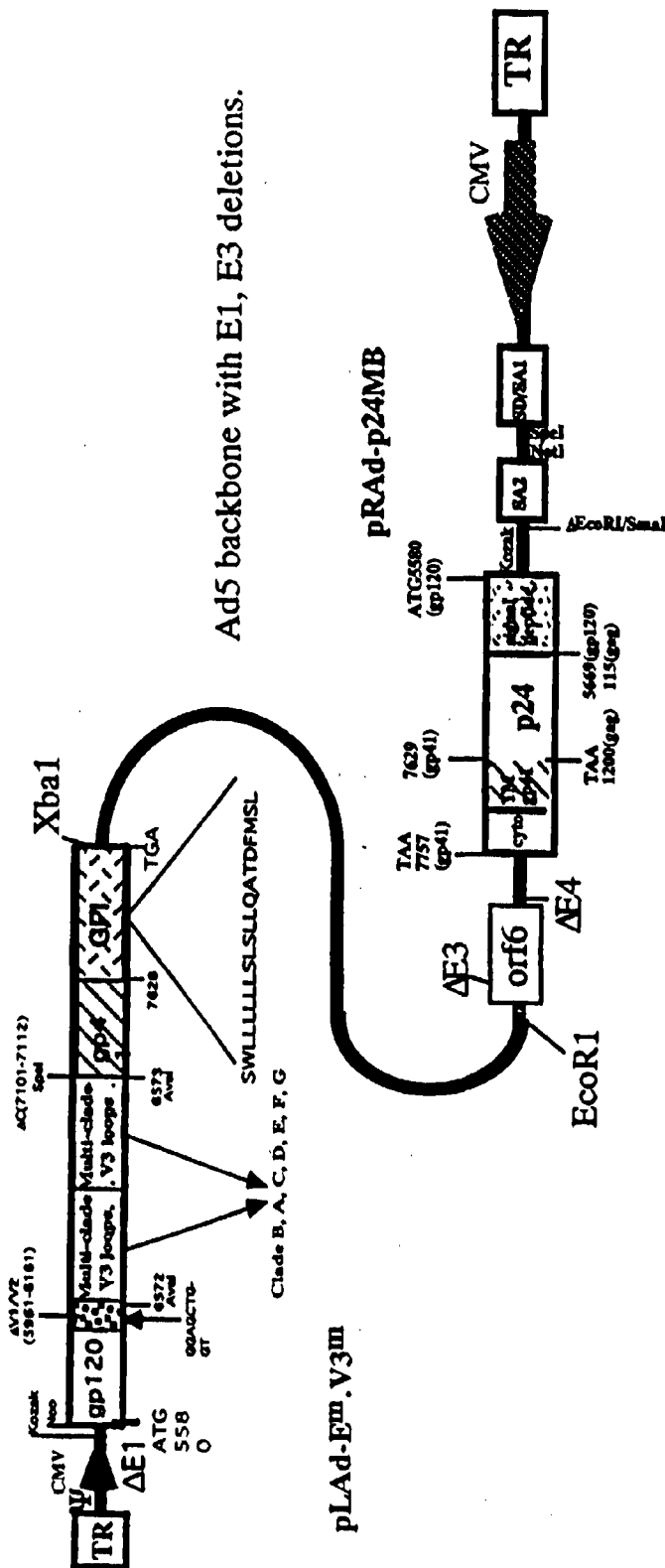


FIGURE 32A

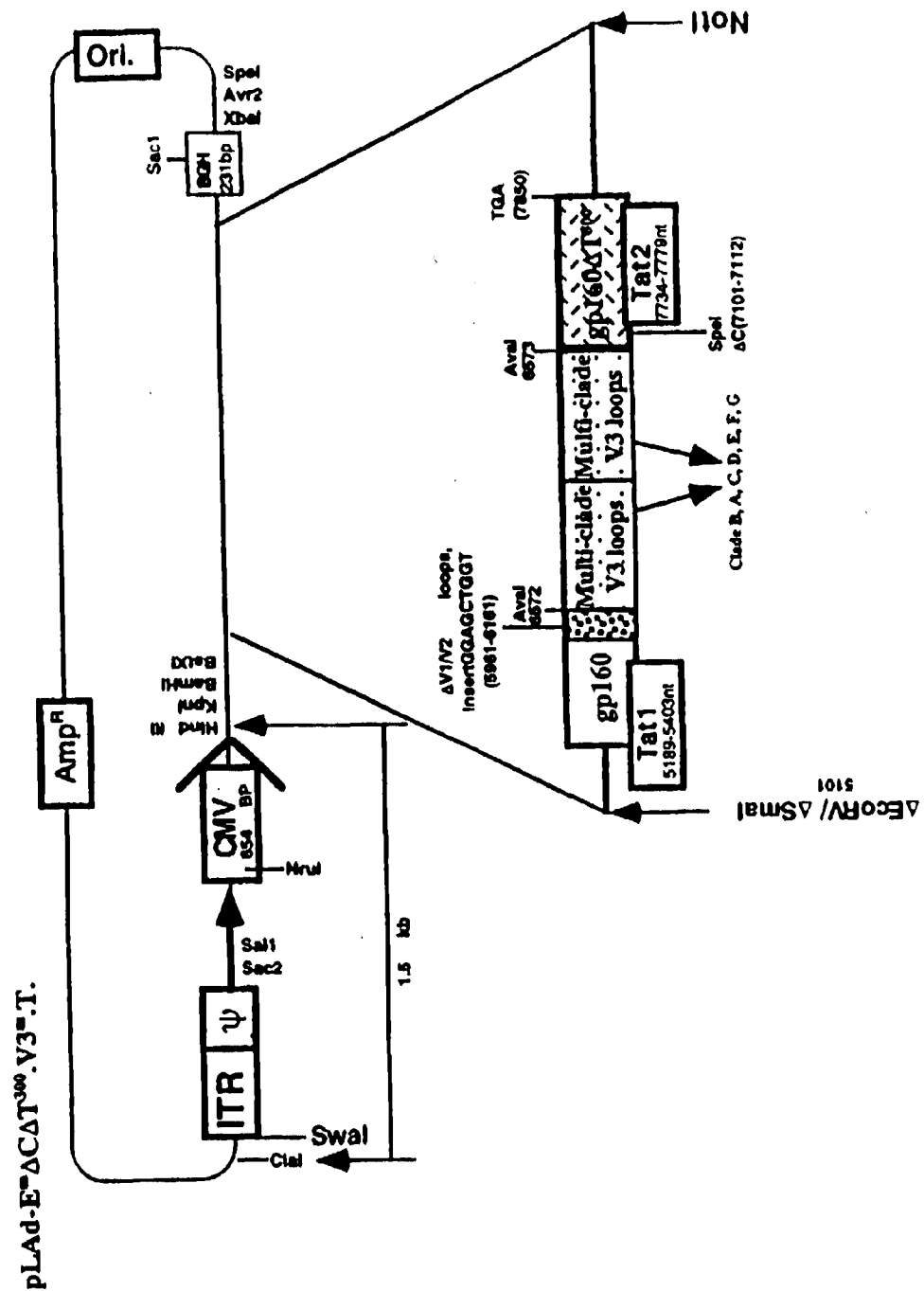




**FIGURE 32B-continued**



**FIGURE 33**



**FIGURE 34**

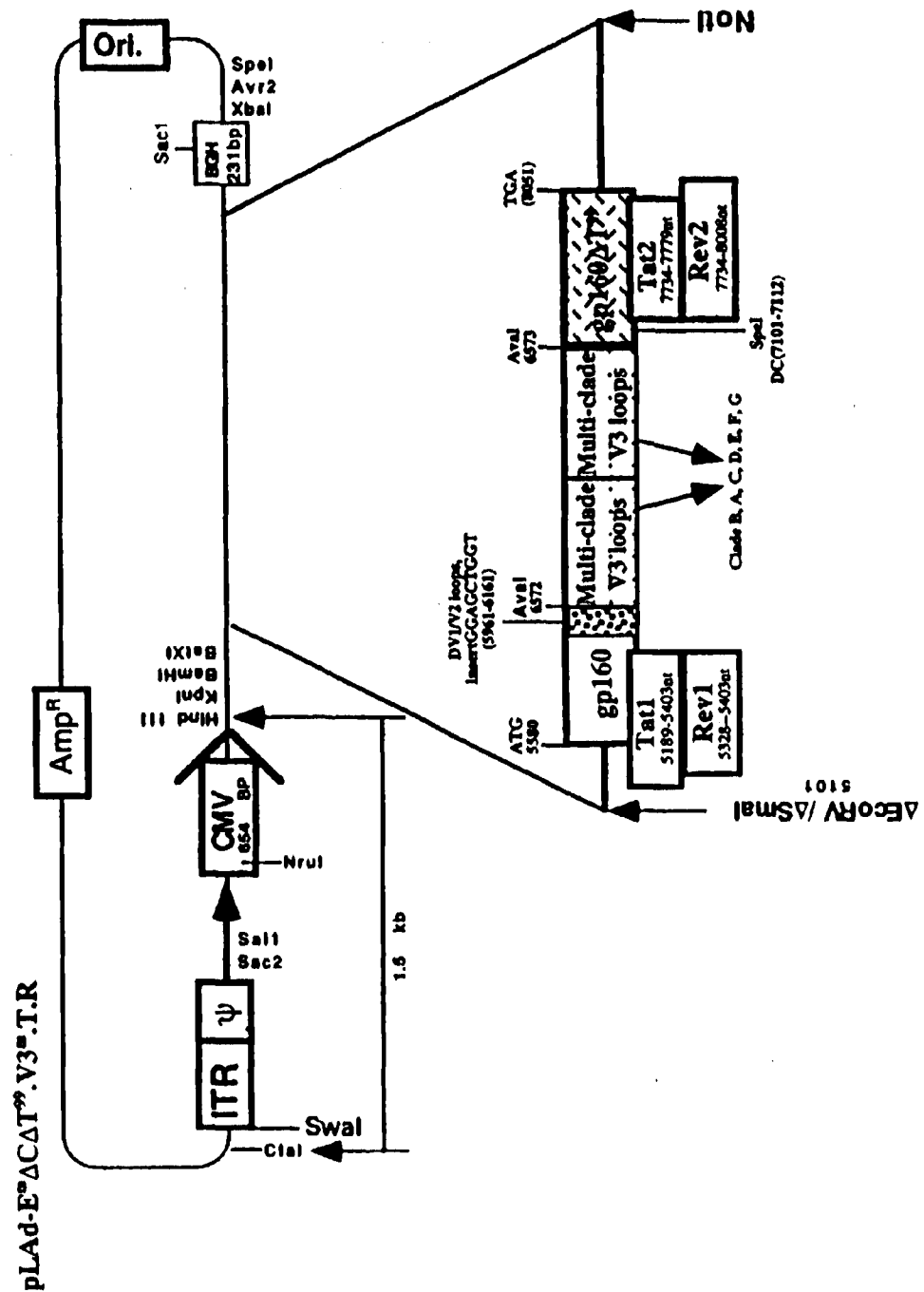




FIGURE 35

pRad. ORF6-G.PI

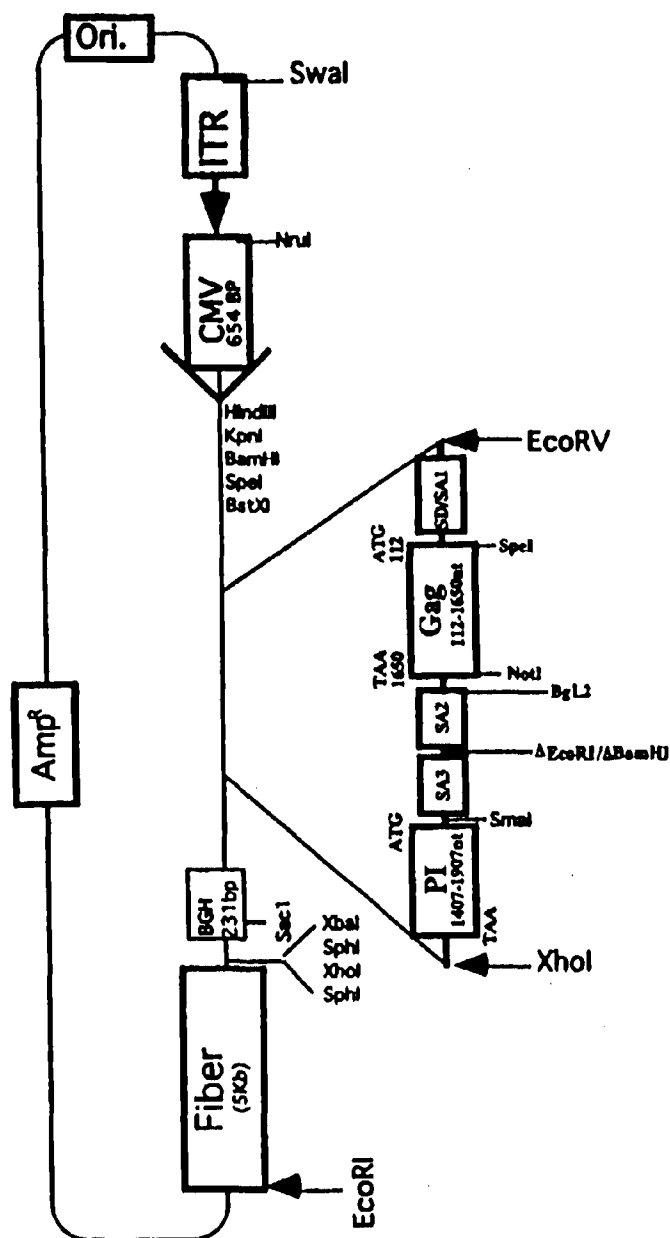


FIGURE 36

pRad.ORF6-G-PI

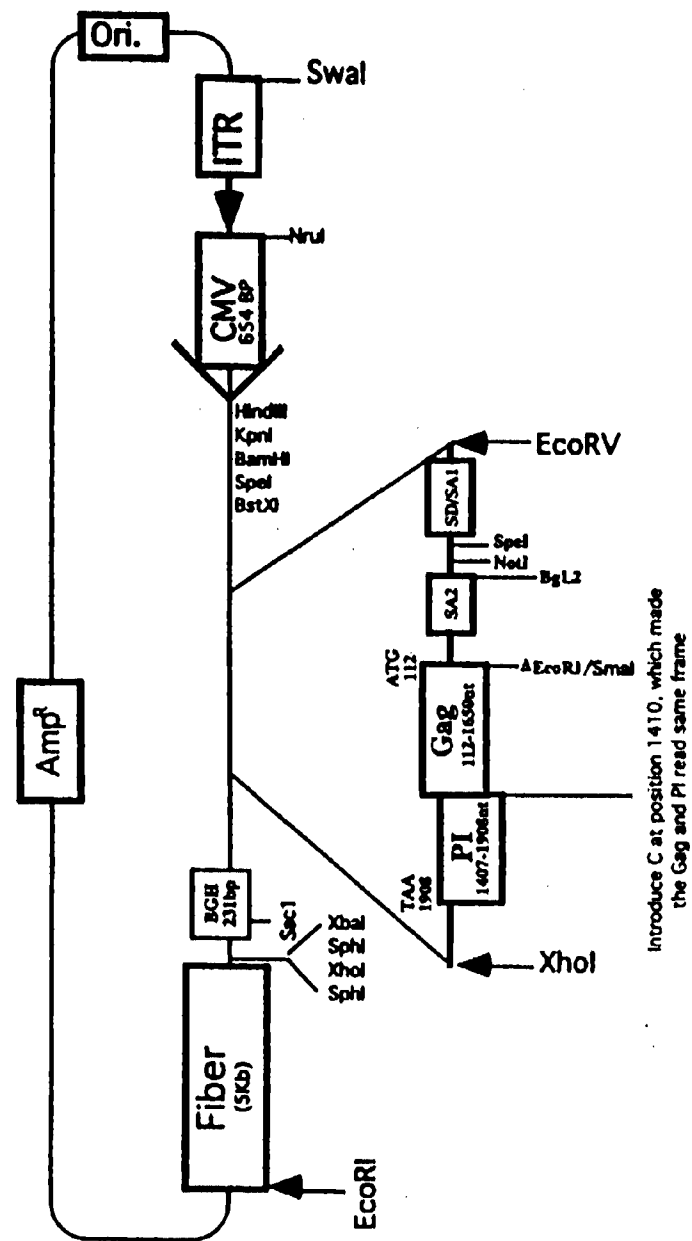
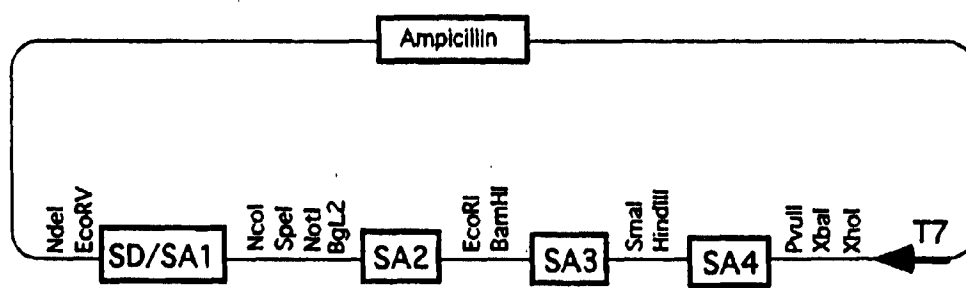


FIGURE 37

SD/SA1.2.3 vector



**FIGURE 38****DNA Sequence of Env/Tat/Rev from BH10 clone (SEQ ID NO: 14):**GaattctgcaacaactgctgtttatccattttcagaattgggtgtcgacatEcoRI

agcagaataggcggttactcgacagaggagagcaagaaatggagccagtagatcctagactagagccctgga  
 agcatccaggaagtcagcctaaaactgcttgtaccaattgctattgtaaaaagtgttgctttcattgccc  
 gtttgtttcatacaaaaagccttaggcatctcctatggcaggaagaagcggagacagcgacgaagacctcc  
 tcaaggcagtcagactcatcaagtttctctatcaaagcagtaagtagtacatgtaatgcaacctatacaaa  
 tagcaatagtagcattagtagtagcaataataatagcaatagttgtgtgggtccatagtaatacatagaatat  
 aggaaaatattaagacaaaagaaaaatagacaggttaattgatagactaatagaaaagcagagaagcagtg  
 caatgagagtgaaaggagaaatatcagcacttgtggagatgggggtggagatggggcaccatgctccttggg  
 atgttgatgatctgtagtgctacagaaaaatgtgggtcacagtcctattatggggtacctgtgtggaagg  
 agcaaccaccactctattttgtgcatcagatgctaaagcatatgatacagaggtacataatgtttgggcca  
 cacatgctgtgtacccacagaccccaaccacagaagtagtatttggtaaatgtgacagaaaaatttaac  
 atgtggaaaaatgacatggtagaacagatgcatgaggatataatcagtttatgggtatcaaagcctaaagcc  
 atgtgtaaaaatcaaccctctgtgttagtttaaagtgcactgatttgaagaatgataactaataccaata  
 gtagtagcgggagaatgataatggagaaaggagagataaaaaactgctctttcaatatcagcacaaagcata  
 agaggttaaggtgcagaaagaatatgcattttttataaaacttgatataataccaatagataatgatactac  
 cagctatacgttgacaagttgtaacacctcagtcattacacaggcctgtccaaaggtatcctttgagccaa  
 ttcccatacattattgtgccccggctgggttttgcgattctaaaatgtaataataagcgttcaatggaaca  
 ggaccatgtacaaatgtcagcacagtagacaatgtacacatggaattaggccagtagtatcaactcaactgct  
 gttaaatggcagctctggcagaagaagaggtagtaattagatctgccaatttcacagacaatgctaaaacca  
 taatagtacagctgaaccaatctgtagaaattaattgtacaagacccaacaacaatacaagaaaaagtatc  
 cgtatccagagaggaccaggagagcatttgttacaataggaaaaataggaaatatgagacaagcacattg  
 taacattagtagagcaaaatggaataacactttaaaacagatagatagcaaatgaagagaacaatttggaa  
 ataataaaaacaataatctttaagcagtcctcaggaggggagccagaaattgtacgcacagttttaattgt  
 ggaggggaatttttctactgtaattcaacacaactgtttaatagtacttgggttaatagtacttggagta  
 ctaaagggtcaataacactgaaggaagtgcacacatcaccctcccatgcagaataaaaacaattataaac  
 atgtggcaggaagtaggaaaagcaatgtatgccccctccatcagtggaacaattagatgttcatcaaatat  
 tacagggtctgctattaacaagagatgggtgtaataagcaacaatgagtcagatcttcagacctggaggag  
 gagatagagggaacaattggagaagtgaattatataaatataaagttagtaaaaattgaaccattaggagta  
 gcaccaccaaggcaagagaagagtggtgcagagagaaaaagagcagtggaataggagcttctgttctc  
 tgggttcttgggagcagcaggaagcactatggcgccagcgtcaatgacgctgacggtacaggccagacaat  
 tattgtctggtatagtcagcagcagaacaatttgcgtgagggtctattgaggcgcaacagcatctgttgcaa  
 ctacagctctggggcatcaagcagctccaggcaagaatcctggctgtggaagatacctaaaggatcaaca  
 gctcctggggatttgggttgcctctggaaaactcatttgcaccactgctgtgccttggaaatgctagtggga  
 gtaataaatctctggaacagatttggaaatacatgacctggatggagtgggacagagaaattaacaattac  
 acaagcttaatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattattgga  
 attagataaatgggcaagtttgggaattgggttaacataacaaattggctgtggtatataaaattattca  
 taatgatagtaggaggttggtaggttaagaatagtttttgcgtgactttctgtagtgaatagagttagg  
 cagggtattaccattatcgtttcagacccacctcccaatcccagggggacccgacaggccgaagggaat  
 agaagaagaaggtggagagagagacagagacagatccattcgattagtgaacggatccttagcactatct  
 gggacgatctgcggagcctgtgcctcttcagctaccaccgcttgagagacttactcttgattgtaacgagg  
 attgtggaacttctgggacgcaggggtgggaagccctcaaatattggtggaatctcctacagttattggag  
 tcaggagctaaagaatagtgcgtgttagcttgcctcaatggcacagctatagcagtagctgaggggacagata  
 ggggttagagaagtagtacaaggagcttatagagctattcgccacatacctagaagaataagacagggcttg  
 gaaaggattttgctataagatgggtggcaagtggtcaaaaagtagtgggtggatggcctgctgtaaggg  
 aaagaatgagacgagctgagccagcagcagatgggtgggagcagcatctcgag

XhoI

**FIGURE 39****DNA Sequence of IL-2ΔX [SEQ ID NO: 15]:**

Tcactctctttaatcactactcacagtaacctcaactcctgccacaatgta  
caggatgcaactcctgtcttgcatcactaagtcttgcaactgtgcacaaa  
cagtgcacctacttcaagttctacaaagaaaacacagctacaactggagca  
tttactgctggatttacagatgattttgaatggaattaataattacaagaa  
tcccaaactcaccaggatgctcacatttaagttttacatgcccaagaaggc  
cacagaactgaaacatcttcagtgcttgaagaagaactcaaacctctgga

ΔXbaI (cta → ctt)

ggaagtgcataaatttagctcaaagcaaaaactttcacttaagacccagggga  
cttaatcagcaatatcaacgtaatagttctggaactaaagggatctgaaac  
aacattcatgtgtgaatatgctgatgagacagcaaccattgtagaatttct  
gaacagatggattaccttttgtcaaagcatcatctcaacactaacttga

**FIGURE 40****DNA Sequence of Env<sup>m</sup>ΔCAT<sup>300</sup> (HIV strain BH10) [SEQ ID NO: 16]:**

**Gaattcgccacccatgggagtggaaggagaaatatcagcacttgtggagatg**  
 EcoRI Kozak NcoI  
 ggggtggagatggggcaccatgctccttgggatgttgatgatctgtagtgtctacagaaaaa  
 ttgtgggtcacagtctattatgggggtacctgtgtggaaggaagcaaccaccactctatctt  
 gtgcatcagatgctaaagcatatgatacagagggtacataatgtttggggccacacatgcctg  
 tgtacccacagaccccaaccacaagaagtagtattggtaaatgtgacagaaaaattttaac  
 atgtggaaaaatgacatggragaacagatgcatgaggatataatcagtttatgggatcaaa  
 gcctaaagccatgtgtaaaattaaccccactctgtgttagtttaaagtgcactgatttgaa  
 gaatgataactaataccaatagtagtagcgggagaatgataatggagaaaggagagataaaa  
 aactgctctttcaatatcagcacaaagcataagaggtaagggtgcagaaagaatatgcatttt  
 ttataaaacttgatataataccaatagataatgatactaccagctatacgttgacaagttg  
 taacacctcagtcattacacaggcctgtccaaagggtatcctttgagccaattcccatacat  
 tattgtgccccggctggttttgcgattctaaaatgtaataataagacgttcaatggaacag  
 gaccatgtacaaatgtcagcacagtacaatgtacacatggaattaggccagtagtatcaac  
 tcaactgctgtttaaattggcagtcctggcagaagaagaggtagtaattagatctgccaatctc  
 acagacaatgctaaaaccataatagtagcagctgaaccaatctgtagaaattaattgtacaa  
 gacccaacaacaatacaagaaaaagtatccgtatccagagaggaccaggaggagagcatttgt  
 tacaataggaaaaataggaaatagagacaagcacattgtaacattagtagagcaaaatgg  
 aataacacttttaaacagatagatagcaaatgaagagaacaatttggaataataaaacaa  
 taatctttaagcagtcctcaggaggggacccagaaattgtaacgcacagttttaattgtgg  
 aggggaatttttctactgtaattcaacacaactgtttaatagtacttggtttaatagtact  
 tggagtactaaagggtcaaataacactgaagggaagtgcacaaatcacccctcccatgcagaa  
 taaaacaaattataaacatgtggcaggaagtaggaaaagcaatgtatgcccctcccatcag  
 tggacaaattagatgttcatcaaatattacagggtgctattaacaagagatgggtggtaat  
 agcaacaatgagtcaggatcttcagacctggaggaggagatatgagggacaattggagaa  
 gtgaattatataaatataaagtagtaaaaattgaaccattaggagtagcaccacccaaggc  
 aaagagaagagtggtgcagACTAGTgcagtggggaataggagctt  
 ΔCleavage site (agagaaaaaga) → SpeI  
 tgttccttgggttcttgggagcagcaggaagcactatggggcgacgcgtcaatgacgctgac  
 ggtacaggccagacaattattgtctggtatagtgcagcagcagaacaatttgctgagggt  
 attgaggcgcaacagcatctgttgcaactcacagtctggggcatcaagcagctccaggcaa  
 gaatcctggctgtggaaagatacctaaaggatcaacagctcctggggatttggggttgcctc  
 tggaaaactcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctg  
 gaacagatttggaataacatgacctggatggagtgggacagagaaattaacaattacacaa  
 gcttaatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaatt  
 attggaattagataaatgggcaagtttgtggaattgggtttaacatacaaatggctgtgg  
 tatataaaattattcataatgatagtaggaggttggtaggtttaagaatagtttttgcctg  
 tactttctgtagtgaatagagtttaggcaggatattcaccattatcgtttcagacccacct  
 cccaatcccagggggacccgacaggcccgaagggaatagaagaagaagggtggagagagagac  
 agagacagatccattcgattagtgaaacggatccttagcacttatctggtaa

**FIGURE 41A****DNA Sequence of Full length HIV-1 Gag [SEQ ID NO: 17]:**

ggctagaaggagagaggatgggtgcgagagcgtcagtattaagcgggggag  
aattagatcgatgggaaaaaattcggttaaggccaggggggaaagaaaaaat  
ataaattaaaacatatagtatgggcaagcaggagctagaacgactacaac  
catcccttcagacaggatcagaagaacttagatcattatataatacagtag  
caaccctctatttgtgtgcatcaaaggatagagataaaagacaccaaggaag  
ctttagacaagatagaggaagagcaaaacaaaagtaagaaaaaagcacagc  
aagcagcagctgacacaggacacagcagtcagggtcagccaaaattacccta  
tagtgcagaacatccaggggcaaattggtacatcaggccatatcacctagaa  
ctttaaatgcatgggtaaaagtagtagaagagaaggctttcagcccagaag  
taatacccatgttttcagcattatcagaaggagccaccccacaagatttaa  
acaccatgctaaacacagtggggggacatcaagcagccatgcaaagttaa  
aagagaccatcaatgaggaagctgcagaatgggatagagtacatccagtgc  
atgcagggcctattgcaccaggccagatgagagaaccaaggggaagtgaca  
tagcaggaactactagtacccttcaggaacaaataggatggatgacaaata  
atccacctatcccagtaggagaaatttataaaagatggataatcctgggat  
taaataaaaatagtaagaatgtatagccctaccagcattctggacataagac  
aaggaccaaagaaccttttagagactatgtagaccggttctataaaactc  
taagagccgagcaagcttcacaggaggtaaaaaattggatgacagaaacct  
tgttggtccaaaatgcgaaccagattgtaagactattttaaagcattgg  
gaccagcggctacactagaagaaatgatgacagcatgtcagggagtaggag  
gaccggccataaggcaagagttttggctgaagcaatgagccaagtaacaa  
atacagctaccataatgatgcagagaggcaatttttaggaaccaaagaaaga  
tggttaagtgtttcaattgtggcaaagaagggcacacagccagaaattgca  
gggcccctaggaaaaagggtgttggaatgtggaaaggaaggacacaaaa  
tgaaagattgtactgagagacaggctaatttttagggaagatctggcctt  
cctacaagggaaggccagggaattttcttcagagcagaccagagccaacag  
ccccaccattttcttcagagcagaccagagccaacagccccaccagaagaga  
gcttcagggtctggggtagagacaacaactccccctcagaagcaggagccga  
tagacaaggaactgtatcctttaacttcctcagatcactctttggcaacg  
accctcgtcacaataaa

**FIGURE 41B****Amino Acid Sequence of HIV-1 (Strain BH10) Gag [SEQ ID NO: 18]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | G | A | R | A | S | V | L | S | G | G | E | L | D | R | W | E | K |
| I | R | L | R | P | G | G | K | K | R | Y | K | L | K | H | I | V | W |
| A | S | R | E | L | E | R | L | Q | P | S | L | Q | T | G | S | E | E |
| L | R | S | L | Y | N | T | V | A | T | L | Y | C | V | H | Q | R | I |
| S | K | K | K | A | Q | Q | A | A | A | D | I | G | H | S | S | Q | V |
| S | Q | N | Y | P | I | V | Q | N | I | Q | G | Q | M | V | H | Q | A |
| I | S | P | R | T | L | N | A | W | V | K | V | V | E | E | K | A | F |
| S | P | E | V | I | P | M | F | S | A | L | S | Q | G | A | T | P | Q |
| D | L | N | T | M | L | N | E | V | A | G | H | Q | A | A | M | P | M |
| L | K | E | T | I | N | E | E | A | A | E | W | D | R | V | H | P | V |
| H | A | G | P | I | A | P | G | Q | M | R | E | P | R | G | S | D | I |
| A | G | T | T | S | T | L | Q | E | Q | I | G | W | M | T | N | N | P |
| P | I | P | V | G | E | I | Y | K | R | I | I | I | L | Q | L | K | K |
| I | V | R | M | Y | S | P | T | S | F | L | D | I | R | G | A | P | Q |
| E | P | F | R | D | Y | V | D | R | T | Y | K | T | L | V | Q | N | A |
| A | S | Q | E | V | K | N | W | M | A | E | T | L | L | A | T | L | E |
| N | P | D | C | K | T | I | L | K | A | L | G | P | A | A | A | R | V |
| E | M | M | T | A | C | Q | G | V | G | T | P | G | H | K | M | Q | R |
| L | A | E | A | M | S | Q | V | T | N | K | A | T | I | M | A | K | E |
| G | N | F | R | N | Q | R | K | M | V | R | C | F | N | C | G | K | C |
| G | H | T | A | R | N | C | K | K | A | T | K | K | G | C | W | F | L |
| G | K | E | G | H | Q | M | K | G | D | P | E | R | Q | A | N | S | R |
| P | K | I | W | P | S | Y | K | L | G | C | G | N | F | L | Q | A | P |
| P | E | P | T | A | P | P | F | V | L | Q | R | P | P | P | T | K | Q |
| E | P | I | S | F | R | S | Y | P | P | T | S | L | R | S | L | F | G |
| N | D | P | S | S | Q | * |   |   |   |   |   |   |   |   |   |   |   |



**FIGURE 42****DNA Sequence of E<sup>m</sup>ΔCΔT<sup>99</sup>.T.R (HIV strain pNL4-3) [SEQ ID NO: 19]:**Gaattctgcaacaactgctgtttatccatttcagaattgggtgtcgacatag

EcoRI

cagaataggcgttactcgacagaggagagcaagaaatggagccagtagatcctagactagagccctggaagca  
 tccaggaagtgcgctaaaactgcttgtaccaattgctattgtaaaaagtgttgccttcattgccaaagtgtt  
 tcatgacaaaagccttaggcatctcctatggcaggaagaagcggagacagcgacgaagagctcatcagaaca  
 gtcagactcatcaagcttctctatcaaagcagtaagtagtacatgtaatgcaacctataatagtagcaatagt  
 agcattagtagtagcaataataatagcaatagttgtgtggtccatagtaatcatagaatataggaaaatatta  
 agacaaaagaaaaatagacagggttaattgatagactaatagaaaagcagagaagacagtggaatgagagtgaag  
 gagaagtatcagcacttgtggagatgggggtggaaatggggcaccatgctccttgggatattgatgatctgta  
 gtgctacagaaaaattgtgggtcacagctctattatgggggtacctgtgtggaaggaagcaaccaccactctatt  
 ttgtgcatcagatgctaaagcatatgatacagaggtacataatgtttgggccacacatgcctgtgtacccaca  
 gaccccaaccacaagaagtagtattggtaaatgtgacagaaaaattttaacatgtggaaaaatgacatggttag  
 aacagatgcatgaggatataatcagtttatgggatcaaagcctaaagccatgtgtaaaatttaacccactctg  
 tgttagtttaagtgactgatttgaagaatgataactaataaccaatagtagtagcgggagaatgataatggag  
 aaaggagagataaaaaactgctctttcaatatcagcacaaagcataagagataaggtgcagaaagaatatgcat  
 tcttttataaacttgatatagtaccaatagataatacca

gctatagggtgataagttgtaacacctcagtcattacacaggcctgtccaaaggtatcctttgagccaattcc  
 catacattattgtgccccggctggttttgcgattctaaaatgtaataataagacgttcaatggaacaggacca  
 tgtacaaatgtcagcacagtagacaatgtacacatggaatcaggccagtagtatcaactcaactgctgttaaatg  
 gcagtcctagcagaagaagatgtagtaattagatctgccaaatttcacagacaatgctaaaaccataatagtaca  
 gctgaacacatctgtagaatttaattgtacaagacccaacaacaatacaagaaaaagtatccgtatccagagg  
 ggaccaggagagcatttgttacaataggaaaaataggaaatagagacaagcacattgtaacattagtagag  
 caaaatggaaatgccactttaaaacagatagctagcaaataaagagaacaatttggaataataaaaacaataat  
 ctttaagcaatcctcaggaggggaccagaaaattgttaacgcacagttttaattgtggagggggaatttttctac  
 tgaattcaacacaactgtttaatagtacttgggtttaatagtacttggagtactgaagggtcaataaacctg  
 aaggaagtgcacacacactcccatgcagaataaaaacaatttataaacatgtggcaggaagtaggaaaaagc  
 aatgtatgccctcccatcagtggaacaaattagatgttcatcaaatattactgggctgctattaacaagagat  
 ggtggtaataacaacaaatgggtccgagatcttcagacctggaggaggcgatatgagggaacattggagaagt  
 aattatataaataataaagtagtaaaaattgaaccattaggagtagcaccaccaaggcaagagaagagtgg  
 gcagACTAGTgcagtggaataggagcttctgttccttg

ΔCleavage site (agagaaaaaga)→SpeI

ggttcttgggagcagcaggaagcactatgggctgcacgtcaatgacgctgacggtacaggccagacaattatt  
 gtctgatatagtgcagcagcagaacaatttgcctgagggctattgagggcgcaacagcatctgttgcaactcaca  
 gtctggggcatcaaacagctccaggcaagaatcctggctgtggaagatacctaaayatacaacagctcctgg  
 ggatttgggggtgctctggaaaactcatttgcaccactgctgtgccttgggaatgctagttggagtaataaaatc  
 tctggaacagatttggaaataacatgacctggatggagtgggacagagaaattaacaattacacaagcttaata  
 cactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaagaattattggaattagataaatggg  
 caagtttgtggaattggtttaacataacaaattggctgtggtatataaaaattattcataatgatagtaggagg  
 ctggtaggtttaagaatagttttgtgtactttctatagtgaatagagttaggcagggtatctcaccatta  
 tcgtttcagacccacctcccaatcccgaggggaccgcaggccgaagggaatagaagaagaaggtggagaga  
 gagacagagacagatccattcgattagtgaacggatccttagcacttatctgggacgatctgcggagcctgtg  
 cctcttcagctaccaccgcttgagagacttactcttgattgtaacgaggattgtggaacttctgggacgcagg  
 ggggtgggaagccctcaaataattggtggaatctcctacagatttgagtcaggaactaaagaatagtgtt  
 acttgcctcaatgccacagccatagcagtagctgagtagaa

**FIGURE 43****DNA Sequence of E<sup>m</sup>ΔV<sub>12</sub>ΔCΔT<sup>99</sup>.T.R (Strain pNL4-3) [SEQ ID NO: 20]:**GaattctgcaacaactgctgtttatccatttcagaattgggtgtcgacatacEcoRI

Cagaataggcgttactcgacagaggagagcaagaaatggagccagtagatcctagactagagccctggaagca  
 tccaggaagtcagcctaaaactgcttgtaccaattgctattgtaaaaagtgttgccttcattgccaagtgtgt  
 ttcattgacaaaagccttaggcatctcctatggcaggaagaagcggagacagcgacgaagagctcatcagaaca  
 gtcagactcatcaagcttctctatcaaagcagtaagtagtacatgtaattgcaacctataatagtagcaatagt  
 agcattagtagtagcaataataatagcaatagttgtgtgtggtccatagtaatcatagaatataggaaaaatatta  
 agacaaagaaaaatagacaggttaattgatagactaatagaaagagcagaagacagtggaatgagagtgaag  
 gagaagtatcagcacttgtggagatgggggtggaatggggcaccatgctccttgggatattgatgactgtga  
 gtgctacagaaaaattgtgggtcacagctctattatggggtacctgtgtggaagggaagcaaccaccactctatt  
 ttgtgcatcagatgctaaagcattatgatacagaggtacataaatgtttgggccacacatgctgtgtaccacca  
 gaccccaaccacagaagtagtatttggtaaatgtgacagaaaaatttaacatgtggaaaaatgacatggttag  
 aacagatgcatgaggatataatcagtttatgggatcaaagcctaaagccatgtgtaaaaattaacccactctg  
 tgtt ΔV1 and V2 loops

Agttgtaacacctcagtcattacacaggcctgtccaaaggtatcctttgagccaattcccatacattattgtg  
 ccccggtgtgttttgcgattctaaaatgtaataataagacgttcaatggaacaggaccatgtacaaatgtcag  
 cacagtacaatgtacacatggaatcaggccagtagtatcaactcaactgctgtttaaattggcagctctagcaga  
 gaagatgtagtaattagatctgccaaatttcacagacaatgctaaaaccataatagtagcagctgaacacatctg  
 tagaaattaattgtacaagacccaacaacaatacaagaaaaagtatccgtatccagaggggaccagggagagc  
 atttgttacaataggaaaaataggaaatatgagacaagcacattgtaacattagtagagcaaaatggaatgcc  
 actttaaaacagatagctagcaaatgaagagaacaatttggaataataaaaacaataatctttaagcaatcct  
 caggaggggacccagaaaattgtaacgcacagttttaattgtggagggggaatttttctactgtaattcaacaca  
 actgtttaatagtacttgggttaatagtacttggagtactgaaggggtcaataaacactgaagggaagtgcaca  
 atcacactcccatgcagaataaaacaatttataaacatgtggcaggaagtaggaaaagcaatgtatgccctc  
 ccatcagtggaacaaattagatgttcatcaaatattactgggctgctattacaagagatgggtggttaatacaaa  
 caatgggtccgagatcttcagacctggaggaggcgatagagggacaattggagaagtgaattatataaatat  
 aaagttagtaaaaaattgaaccattaggagtagcaccaccaaggcaagagaagagtggtgcagACTAGTgcag  
 tgggaataggagctttgttccttgggttcttgggagca

ΔCleavage site (agagaaaaaga) → SpeI

gcaggaagcactatgggctgcacgtcaatgacgctgacgggtacaggccagacaattattgtctgatatagtcg  
 agcagcagaacaatttgcctgagggctattgagggcgcaacagcatctgttgcaactcacagtctggggcatcaa  
 acagctccaggcaagaatcctggctgtggaagatacctaaaggatcaacagctcctggggatttgggggtgc  
 tctggaaaactcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctggaacagattt  
 ggaataacatgacctggatggagtgggacagagaaatgaacaattacacaagcttaatacactccttaattga  
 agaatcgcaaaaccagcaagaaaagaatgaacaagaattattggaattagataaatgggcaagtttgtggaat  
 tgggttaacataacaaattggctgtggtatataaaattattcataatgatagtaggaggttggtaggtttaa  
 gaatagtttttgcctgacttctatagtgaatagagtttaggcagggatattcaccattatcggttcagaccca  
 cctcccaatcccaggggacccgacagggcccgaaaggaatagaagaagaaggtggagagagagacagagacaga  
 tccattcgattagtgaaacggatccttagcacttatctgggacgatctgcggagcctgtgcctcttcagctacc  
 accgcttgagagacttactcttgattgtaacgaggattgtggaactctgggacgcaggggggtgggaagccct  
 caaataattggtggaatctcctacagtagtgagtcagggaactaaagaatagtgctgttaactgtcgaatgcc  
 acagccatagcagtagctgagtaa

**FIGURE 44**

**DNA Sequence of Env<sup>m</sup>ΔC.T.R.N (Strain BH10) [SEQ ID NO: 21]:**

Gaattctgcaacaactgctgtttatccatttcagaattgggtgtcgacat

**EcoRI**

agcagaataggcggttactcgacagaggagagcaagaatatggagccagragatcctagactagagcctctgga  
 agcatccaggaagtcaagcctaaaactgcttctgaccaattgctattgtaaaaagtgttgctttcatttgccaa  
 gtttgtttcataacaaaagccttaggcattctctatggcaggaagaagcggagacagcgacgaagacacctcc  
 tcaaggcagtcagactcatcaagttctctatacaaacagtagtaagtgtacatgtaatgcaacctatagaatat  
 tagcaatagctagcattagtagtagcaataataatagcaaatagttgtgtggtccatagtaaatcatagataaat  
 aggaaaaatattaagacaaaagaaaaatagacagggttaattgtatagactaatagaaaagagcagaagacagtggtg  
 caatgagagtggaaggagaaaatatcagcacttgtggagatgggggtggagatggggcaccatgctccttggg  
 atgttgatgactctgtagtgctacagaaaaaatgtgggtcacagctctattatgggggtacctgtgtggaagga  
 agcaaccaccactctattttgtgcatcagatgctaaagcatatgatcacagaggtacataatgtttggggcca  
 cacatgctgtgtacctccacagaccccccaagaaagtagtatgtgtaaatgtgacagaaaaatttaac  
 atgtggaaaaatgacatggtagaacagatgcargaggatataatcagtttatgggatcaaagcctaaagcc  
 atgtgtaaaaattaacccactctgtgttagtttaagtgcactgatttgaagaatgatactaataccaata  
 gtatgtagcgggagaaatgataatggagaaaggagagataaaaaactgctctttcaatatcagcaaacgata  
 agaggttaaggtgcagaagaagaatgcatttttataaaactgtatataaccaatagataatgatatactac  
 cagctatacgttgacaagttgtaaacctcagtcattacacaggctctgccaaagggtatcctttgagccaa  
 ttcccatacattattgtgccccggctggttttgcatcttaaaatgtaataataagacgttcaatggaaca  
 ggaccatgtacaaatgtcagcacagtcacaatgtacacatggaattaggccagtatgatcaactcaactgct  
 gttaaattggcagctctggcagaagaagaggtagtaattagatctgccaaatttcacagacaaatgtaaaaacca  
 taatagtacagctgaaccaatctgtagaataatgtacaagaccacaacaaatagaaaagaaatgatc  
 cgtatccagagaggaccaggagagcatttgcataataggaaaaataggaaatatgagacaagcacattg  
 taacattagtagagcaaaaatggaataaacactttaaaacagatagatagcaaatagagagaacaatttgga  
 atataaaaacaataatctttaagcagctctcaggaggggaccagaaaattgtaacgcacagttttaattgt  
 ggaggggaaattttctactgtaatccaacacaaactgtttaatagctacttggtttaatagtactttgagtagc  
 taaagggctcaataaacactgaaggagtgcacaactcaccctccatcgagaataaaaacaattataaaca  
 tgtggcaggaaagtaggaaaaagcaattgtgccctcccatcagtgggacaaattagatgttcatcaaatatt  
 acagggctgctattacaagagatggtggttaatgcaacaatgagtcggagatcttcagacctggaggagg  
 agatatgagggacaattgggagaagtgaattatataaataaaagtgtaaaaattgaaccattaggagtag  
 caccaccaaggcaaaagagaagagtgtgcaagACTAGTgcagtggaataggagctttgttctctgggttc  
 t

ΔCleavage site (acagaaaaaaga)→SpeI

tgggagcagcaggaagcactatggtggcgacgctcaatgacgctgacggtagcaggccagacaattattgtct  
 ggtatagtgcagcagcagaacaatttgtctgagggctattgaggcgcaacagcatctgttgcaactcacagt  
 ctggggcatcaagcagctccaggcaagaatcctggctgtggaaagatacctaaggatcaacagctcctgg  
 ggatttggggttgcctctggaaaaactcatttgcaccactgctgtgccttggaaatgctagtgtggagtaataaa  
 tctctggaaacagatttggaaatacatgacctggatggagtgggacagagaaattacaattacacaagctt  
 aatacactccttaattggaagaatcgcaaaaccagcaagaaaagaatgaacaagaattattggaattagata  
 aatgggcaagtttgtggaattggtttaacataacaatttggctgtggtatataaaatttctcataattgata  
 gtaggaggccttggtaggtttaagaatagtttttgcctgtacttctgtagtgaatagagttaggcagggata  
 ttaccattatcgtttcagaccacctcccaatcccgaggggacccgcagggccgaaggatagaagaag  
 aaggtggagagagagacagagacagatccattcgattagtgaacggatccttagcacttattctgggacgat  
 ctgcgagcctgtgcctcttcagctaccaccgcttgagagacttactcttgattgtaacgaggatttggga  
 acttctgggacgcagggggtgggaagccctcaaatttggggaatctctacagatttggagtcaggagc  
 taagaataagtctgtttagcttgcctcaatgcacagctatagcagttagctgaggggacagatagggttata  
 gaagttagtaacaaggagcttatagagctatttcgccacatacctagaagaataagacagggcttggaaaggat  
 tttgctataagatgggtggcaagtggtaaaaagttagtgtggttggatggcctgctgtaagggaagaagt  
 agacgagctgagccagcagcagatggggtgggagcagcatctcgagacctagaaaaacatggagcaatcac  
 aagtagcaacacagcagctaacaaatgctgatttgcctggctagaaagcacaaggaggaggaggtgggt  
 ttccagtcacacctcaggtacctttaagaccaatgacttacaaggcagctgtagatttagccactttta  
 aaagaagaagggggactggaaggctaatcactcccaacgaagacaagatatccttgatctgtggtatcta  
 ccacacacaaaggctacttccttgattag

**FIGURE 45****DNA Sequence of E<sup>m</sup>ΔC.N (Strain BH10) [SEQ ID NO: 22]:**

Gaattcgccaccatgggagtgaggagaaaatcacgcacttgtggagatgg  
 EcoRI Kozak NcoI  
 ggggtggagatggggcaccatgctccttgggatgttgatgatctgtagtgtctacagaaaaattgtgggtcac  
 agtctattatggggtacctgtgtggaaggaagcaaccaccactctattttgtgcatcagatgctaaagcat  
 atgatacagaggtacataaatgtttgggcccacacatgcctgtgtaccacagaccccaaccacaagaagta  
 gtattggtaaatgtgacagaaaaatttaacatgtggaaaaatgacatggtagaacagatgcatgaggatat  
 aatcagtttatgggatcaaaagcctaaagccatgtgtaaaattaaacccactctgtgttagtttaaagtgc  
 ctgatttgaagaatgataactaataccaatagtagtagcgggagaatgataatggagaaaaggagagataaaa  
 aactgctctttcaatatcagcacaaagcataagaggttaagggtgcagaaagaatatgcattttttataaact  
 tgatataataccaatagataatgatactaccagctatacgttgacaagttgttaacacctcagtcattacac  
 aggcctgtccaaaaggatcctttgagccaattcccatcacattattgtgccccggctgggttttgcgattcta  
 aaatgtaataataaagcgttcaatggaacaggaccatgtacaaatgtcagcacagtacaatgtacacatgg  
 aattagggcagtagtatcaactcaactgctgttaaatggcagctctggcagaagaagggttagtaatttagat  
 ctgccaatttcacagacaatgctaaaaccataatagtacagctgaaccaatctgtagaaattaattgtaca  
 agaccaacaacaatacaagaaaaagtatccgtatccagagaggaccaggaggagcatttgttacaatagg  
 aaaaataggaaatatgagacaagcacattgttaacattagtagagcaaaatggaataaacactttaaaacaga  
 tagatagcaaatgaagagaacaatttggaaataataaaacaataatctttaagcagtcctcaggaggggac  
 ccagaaattgttaacgcacagttttaattgtggaggggaatttttctactgttaattcaacacaactgttta  
 tagtacttgggttaataatgacttggagtagtaaaagggccaataaacactgaagggaagtgcacaatcccc  
 tccatgcagaataaaaacaattataaacatgtggcaggaagtaggaaaagcaatgtatgccccctccatc  
 agtggacaaattagatgttcatcaaatattacagggctgctatttaacaagagatgggtggtaatagcaaca  
 tgagtcagagatcttcagacctggaggaggagatatgagggacaattggagaagtgaattatataaatata  
 aagtagtaaaaattgaaccattaggagtagcaccaccaaggcaagagaagagtggtgcagACTAGTgca  
 gtgggaataggagcttctgtccttgggttcttgggagc  
 ΔCleavage site(agagaaaaaga)→SpeI  
 agcaggaaagcactatgggcgcagcgtcaatgacgtgacggtacaggccagacaattattgtctgggtatag  
 tqcagcagcagaacaatttgcgtgagggctattgagggcgaacagcatctgttgcaactcacagtcctggggc  
 atcaagcagctccaggcaagaatcctggctgtggaagatacctaaaggatcaacagctcctggggatttg  
 ggggtgtctctggaaaactcatttgcaccactgctgtgccttgggaatgctagttggagtaataaatctctgg  
 aacagatttggaaataacatgacctggatggagtgaggacagagaaattacaattacacaagcttaatacac  
 tccttaattgaagaatcgcaaaaccagcaagaaaaagaatgaacaagaattattggaattagataaatgggc  
 aagtttgtggaattgggttaacataacaaattggctgtggtatataaaattattcataatgatagtaggag  
 gcttggtaggtttaagaatagttttgtctgtactttctgtagtgaatagagttaggcagggtatctacca  
 ttatcgtttcagacccacctcccaatcccagggggacccgacaggcccgaaaggaatagaagaagaagggtgg  
 agagagagacagagacagatccattcgattagtgaacggatccttagcacttatctgggacgatctgcgga  
 gctgtgcctcttcagctaccaccgcttgagagacttactcttgattgtaacgaggattgtggaaactttcg  
 ggacgcaggggggtgggaagccctcaaatattgggtggaatctcctacagtattggagtcaggagctaaagaa  
 tagtgcgttagcttgcataatgccacagctatagcagtagctgaggggacagatagggttatagaagtag  
 tacaaggagcttatagagctattcgccacatacctagaagaataagacagggcttggaaaggattttgcta  
 taagatgggtggcaagtgggtcaaaaagttagtgggttggtggcctgctgtaaggggaagaatgagacgag  
 ctgagccagcagcagatgggggtgggagcagcatctcgagacctagaaaaacatggagcaatcacagtagc  
 aacacagcagctaacaatgctgattgtgcctggctagaagcacaagaggaggaggtgggtttccagt  
 cacacctcaggtacctttaagaccaatgacttacaaggcagctgtagatcttagccactttttaaaagaaa  
 aggggggactggaagggttaattcactcccaacgaagacaagatatccttgatctgtggatctaccacaca  
 caaggctacttccctgattag

**FIGURE 46****DNA Sequence of E<sup>m</sup>ΔCΔT<sup>300</sup>.T (BH10) [SEQ ID NO: 23]:**

Gaattctgcaacaactgctgtttatccatcttcagaattgggtgtcgacat  
 EcoRI

Agcagaataggcggttactcgacagaggagagcaagaaatggagccagtaga

Tat 1

tcctagactagagccctggaagcatccaggaagtgcgcctaaaaactgcttgtaaccaattgctattgtaaaa  
 agtgttgctttcattgccaagtttgtttcataacaaaagccttaggcattctcctatggcaggaagaagcgg  
 agacagcgacgaagacctcctcaaggcagtcagactcatcaagtttctctatcaaagcagtaagtagtaca  
 tgtaaatgcaacctatacaaatagcaatagtagcattagtagtagcaataataatagcaatagttgtgtggt  
 ccatagtaaatcatagaatataggaataatattaagacaaaagaaaaatagacaggttaattgatagactaata  
 gaaagagcagaagacagtggaatgagagtggaaggagaaatatacagcacttggtggagatgggggtggagat  
 ggggcaccatgctccttgggatgttgatgactgttagtgctacagaaaaatttggtggtcacagtcctattat  
 ggggtacctgtgtggaagggaagcaaccaccactctattttgtgcatcagatgctaaagcatatgatacaga  
 ggtacataatgtttgggcccacacatgctgtgtacccacagaccccaaccacaagaagtagtattggttaa  
 atgtgacagaaaaattttaacatgtggaataatgacatggtagaacagatgcatgaggatataatcagttta  
 tgggatcaaagcctaaagccatgtgtaaaattaaacccactctgtgttagtttaaagtgcactgatttgaa  
 gaatgatactaataccaatagtagtagcgggagaatgataatggagaaaggagagataaaaaactgctctt  
 tcaatatcagcacacaagcataagaggtgaaggtgcagaaagaatatacattttttataaacttgatataata  
 ccaatagataatgatactaccagctatacgttgacaagttgtaacacctcagtcattacacagggcctgtcc  
 aaaggtatcctttgagccaattcccatacattattgtgccccgctggttttgctgattctaaaatgtataa  
 ataagacgttcaatggaacaggaccatgtacaaatgtcagcacagtagcaatgtacacatggaattaggcca  
 gtagtatcaactcaactgctgttaaatggcagctctggcagaagaagaggtagtaatttagatctgccaattt  
 cacagacaatgctaaaaccataaatagtagcagctgaaccaatctgtagaataatgtacacagacccaaca  
 acaatacaagaaaaagtatccgtatccagagaggaccagggagagcatttgttacaataggaataatagga  
 aatatgagacaagcacattgttaacattagtagagcaaaatggaataaacactttaaaacagatagatagcaa  
 attaagagaacaatttggaaataataaaacaataatctttaagcagtcctcaggaggggacccagaaattg  
 taacgcacagttttaattgtgaggggaatttttctactgttaattcaacacaactgtttaatagtagtctgg  
 tttaatagtagtacttgagtagtactaaagggtcaataaacactgaagggaagtgcacacatcacctcccatgcag  
 aataaaacaaattataaacatgtggcaggaagtaggaaaagcaatgtatgcccctcccatcagtggaacaaa  
 ttagatgttcatcaaatattacagggctgctatttaacaagagatggtggttaatagcaacaatgagtcagag  
 atcttcagacctggagaggagatatagagggacaattggagaagtgaattatataaatataaagtagtaaa  
 aattgaaccattagtagtagcaccaccaaaggcaagagaagagtggtgcagACTAGTgcagtggaataag  
 gagctttgttccttgggttc

ΔCleavage site (agagaaaaaga) → SpeI

ttgggagcagcaggaagcactatgggcgcagcgtcaatgacgctgacggtacagggccagacaattattgtc  
 tggtagtagtcagcagcagaacaatttgcaggggtattgagggcgaacagcatctgttgcaactcacag  
 tctggggcatcaagcagctccaggcaagaatcctggctgtggaagatacctaaggatcaacagctcctg  
 gggatttgggttgctctggaactcatttgaccactgctgtgccttgggaatgtagttggagtaataa  
 atctctggaacagatttggaaataacatgacctggatggagtgaggacagagaaattaacaattacacaagct  
 taatacactccttaattgaagaatcgcaaaaccagcaagaaaaagaatgaacaagaattattggaattagat  
 aaatgggcaagtttgggaattggtttaacataacaaattggctgtggtatataaaattattcataatgat  
 agtaggaggttggtaggtttaagaatagtttttgcgtgactttctgtagtgaatagagtttaggcagggat  
 attcaccattatcgtttcagacccacctcccaatcccaggggacccgacagggcccgaggaatagaagaa  
 gaaggtggagagagagcagagacagatccattcgattagtgaaacggatccttagcacttatctggttaa

**Figure 47****DNA Sequence of E<sup>m</sup>/E<sup>m</sup> (BH10) [SEQ ID NO: 24]:**

Gaattcgccaccatgggagtgaggagaaaatcagcacttctggagatcg  
 EcoRI Kozak NcoI

gggtggagatggggcaccatgctccttgggatgttgatgatctgtagtgctacagaaaaatttgggtcac  
 agtctattatggggtacctgtgtggaagggaagcaaccaccactctattttgtgcatcagatgctaaagcat  
 atgatacagaggtacataaatgtttgggccacacatgcctgtgtacccacagaccccaaccacaagaagta  
 gtattggtaaatgtgacagaaaaatttaacatgtggaaaaatgacatggtagaacagatgcatgaggatat  
 aatcagtttatgggatcaaagcctaaagccatgtgtaaaatttaacccactctgtgttagtttaaagtgc  
 ctgatttgaaagaatgatactaataccaatagtagtagcgaggagaatgataatggagaaaggagagataaaa  
 aactgctctttcaatatcagcacaaagcataagaggtaagggtgcagaaagaatatgcattttttataaact  
 tgatataataaccaatagataaatgatactaccagctatacgttgacaagttgttaacacctcagtcattacac  
 aggcctgtccaaagggtatcctttgagccaattcccatacattattgtgccccggctgggttttgcgattcta  
 aaatgtaataataagacgttcaatggaacaggaccatgtacaaatgtcagcacagtaaatgtacacatgg  
 aattaggccagtagtatcaactcaactgctgttaaatggcagctctggcagaagaagggtagtaatttagat  
 ctgccaatttcacagacaatgctaaaaccataatagtacagctgaaccaatctgtagaatttaattgtaca  
 agaccaacaacaatacaagaaaaagttatccgtatccagagaggaccaggaggagagcatttgttacaatagg  
 aaaaataggaaatatgagacaagcacattgtaacattagtagagcaaaatggaataaacacttttaaacaga  
 tagatagcaaatgaagagaacaatttggaataataaaacaataatctttaagcagtcctcaggagggggac  
 ccagaaattgtaacgcacagttttaaattgtggagggggaatttttctactgtaattcaacacaactgtttaa  
 tagtacttggtttaatatgtacttggagtactaaaggggtcaataaacactgaagggaagtgcacaatcaccc  
 tcccatgcagaataaaaacaattataaacatgtggcagggaagttaggaaaagcaatgtatgccctcccatc  
 agtggacaaaattagatgttcataaatattacagggtctgctattaacaagagatggtggtaataagcaaaa  
 tgagtccgagatcttcagacctggaggaggagatatgaggggacaattggagaagtgaattatataaatata  
 aagtagtaaaaaattgaaccattaggagtagcaccaccaaggcaagagaagagtggtgcagagagaaaaa  
 agagcagtggggaataggagctttgttccttgggttcttgggagcagcaggaagcactatgggcgcagcgtc  
 aatgacgctgacggtacagggccagacaattattgtctgggtatagtgcagcagcagacaatttgcagggg  
 ctattgaggcgcaacagcatctgttgcaactcacagctctggggcatcaagcagctccaggcaagaatcctg  
 gctgtggaaagatacctaaaggatcaacagctcctggggatttgggggtgctctggaaaactcatttgcac  
 cactgctgtgccttgggaatgctagttggagtaataaatctctggaacagatttggaaataacatgacctgga  
 tggagtgggacagagaaattaacaattacacaagcttaatacactccttaattgaagaatcgcaaaaccag  
 caagaaaaagaatgaacaagaattattggaattagataaatgggcaagtttgggaatttgggttaacataac  
 aaattggctgtggtatataaaattattcataatgatagtaggaggcttggtaggtttaagaatagtttttg  
 ctgtactttctgtagtgaatagagtttaggcagggatattcaccattatcgtttcagacccacctcccaatc  
 ccgaggggacccgacagggcccgaaaggaatagaagaagaaggtggagagagagacagagacagatccattcg  
 attagtgaacggatccttagcacttatctgggacgatctgcggagcctgtgcctcttcagctaccaccgct  
 tgagagacttactcttgattgttaacgaggattgaggaaacttctgggacgcagggggtgggaagccctcaa  
 tattgggtggaatctcctacagttatggagtcaggagctaaagaatagtgtgttagcttgctcaatgccac  
 agctatagcagtagctgaggggacagatagggttatagaagtagtacaaggagcttatagagctatttcgcc  
 acatacctagaagaataagacagggttggaaaggattttgctataa

**FIGURE 48****Sequences of V3 loop Multi-clade HIV-1 Clones:**

| Clade | ACC#   | HIV-1 Strain         | From(nt) | To(nt) |
|-------|--------|----------------------|----------|--------|
| B     | M15654 | BH10                 | 885      | 992    |
| A     | U09127 | 192UG037WHO.01083hED | 888      | 992    |
| C     | U09126 | 192BR025WHO.01093hED | 876      | 980    |
| D     | U43386 | 192UG024.2           | 888      | 989    |
| E     | U08458 | 193TH976.17          | 894      | 998    |
| F     | U27401 | 193BR020.17          | 888      | 992    |
| G     | U30312 | 192RU131.9           | 885      | 989    |

Tgtacaagacccaacaacaataacaagaaaaagtatccgtatccagagagga  
ccaggagagcatttgttacaataggaaaaataggaaatatgagacaagca  
cattgt **Clade B [SEQ ID NO: 25]**

Tgtaccagacctaacaacaataacaagaaaaagtgtacgtataggaccagga  
caaacattctatgcaacagggtgatataataggggatataagacaagcacat  
tgt **Clade A [SEQ ID NO: 26]**

Tgtacgagacccaacaataataacaagaaaaagtataaggataggaccagga  
caagcattctatgcaacaggagaaataataggagatataagacaagcacat  
tgt **Clade C [SEQ ID NO: 27]**

Tgcacaaggccctacaacaataataagacaaaggacccccataggactaggg  
caagcactctataacaagaagaatagaagatataagaagagcacattgt  
**Clade D [SEQ ID NO: 28]**

Tgtaccagaccctccaccaataacaagaacaagtatacgtataggaccagga  
caagtattctatagaacaggagacataacaggagatataagaaaagcatat  
tgt **Clade E [SEQ ID NO: 29]**

Tgtacaagacccaacaacaataacaagaaaaagaatatctttaggaccagga  
cgagtattttatacagcaggagaaataataggagacatcagaaaggcacat  
tgt **Clade F [SEQ ID NO: 30]**

Tgtaccagacctaataacaataacaagaaaaagtataacttttgcaccagga  
caagcgctctatgcaacagggtgaaataataggagatataagacaagcacat  
tgt **Clade G [SEQ ID NO: 31]**

## FIGURE 49A

**DNA sequence of modified Env including multi-clade V3 loops [SEQ ID NO: 32]:**

Atgagagtgaaaggagaaatatcagcacttctggagatgggggtggagatggggcaccatgctccttgggat  
gttgatgatctgtagtgctacagaaaaatttgggtcacagtctattatgggtacctgtgtggaaggaag  
caaccaccactctatttctgcatcagatgctaaagcatatgatcacagaggtacataatgtttggggcaca  
catgcctgtgtacccacagaccccaaccacagaagtagtattggtaaattgtgtacagaaaaatttaacat  
gtgaaaaaatgacatggtagaacagatgcatgaggataatacagttttatgggatcaaagcctaaagccat  
ctgtaaaaattaacccactctgtgttggagctgggttagttgttaacacctcagt

V1, V2 deletion, GAG insertion

Gattacacaggcctgtccaaagggtatcctttgagccaattcccatacattattgtgcccggtgggtttg  
cgattctaaaatgtaataataagacgttcaatggaacaggaccatgtacaaatgtcagcacagtacaatgt  
acacatggaattaggccgtagtatcaactcaactgctgttaaatggcagctctggcagaagaagggtagt  
aattagatctgcgaatttcacagacaatgctaaaaccataatagtacagctgaaccaatctgtgaaatta  
atttacaagacccaacaaca

Start of Clade B

Tacaagaaaaagtatccgatatccagagaggaccagggagagcatttgttacaataggaaaaataggaaata  
tgagacaagcacattgtctcgggtgtaccag

Insert a Avail site      Clade A

Acctaacacaacaatacagaagaaaaagtgtacgtataggaccaggacaaacattctatgcaacaggtgatataa  
taggggatataagacaaagcacattgtgtgtac

Clade C

Gagaccaacaataatacaagaaaagtataaggataggaccaggacaagcattctatgcaacaggagaaa  
taataggagatataagacaagcacattgttg

Clade D

Cacaaggccctacaacaatataagacaaagacccccataggactagggcaagcactctatacaacaagaa  
gaatagaagatataagaagagcacattgttg

Clade E

Taccagaccctccaccaatacaagaacaagtatacgatataggaccaggacaagtatctctatagaacaggag  
acataacagggatataagaaaagcatattgtggactctgttacaagacccaacaacaatacaagaaaaaga  
atatctttagg

BamHI clade F

AccaggacgagtatTTTTATACAGCAGGAGAAATAATAGGAGACATCAGAAAGGCACATTGTTGTACCAGAC  
CTAATAACAATACAAGAAAAAGTATAACTTT

Clade G

Tgcaccaggacaagcgctctatgcaacaggtgaaataataggagatataagacaagcacattgtctcggga  
acattagtagagcaaatggaataaacacttt

Insert a Avail

Aaaacagatagatagcaaaattaaagagaacaatttggaaataataaaaacaataatctttaagcagtcctcag  
gaggggaccagaaattgtaacgcacagtttttaattgtggagggggaatttttctactgttaattcaacacaa  
ctgtttaatagtacttggtttaatagtacttggagtactaaagggcacaataacactgaaggaagtgcac  
aatcacctcccatgcagaataaaacaaattataaacatgtggcaggaagttagggaaaagcaatgtatgcc  
ctcccatcagtggaacaaattagatgttcatcaaataattacagggctgctattaaacaagagatggtgtaat  
agcaacaatgagtcagatcttcagacctggaggaggagatatgagggacaattggagaagtgaattata  
taataataaagtagtataaaattgaaccattaggagtagcaccaccaaggcacaagagaagagtgggtgcaga  
ctaotcagtggg

Cleavage site mutation (SpeI)

Aataggagcttctgtcctctgggtctctgggagcagcaggaagcactatgggcgcagcgtcaatgacgctga  
 cgggtacaggccagacaattattgtctgtgtatagtgcagcagcagacaatttgcctgagggcctattgaggcg  
 caacagcatctgttgcaactccagctctggggcatcaagcagctccaggcaagaattcctgctgtggaagag  
 atacctaaaggtacacagctctggggatttggggctgctctggaaaactcatgtgcacctgctgtgtgc  
 ctggaaatgctagtgtggagtaataaattctctggaacagatttggaaataacatgacctggatggagtgggac  
 agagaaattaacaattacacaaagcttaatacactccttaattgaaagaatcgcaaaaccagcaagaaagaa  
 tgaacagaagaattattggaattagataaattgggcaagtttgtggaattggtttaacataacaaattggctgt  
 ggtatataaaaatcgtggctgtctgtctctctcctctcctcctcctcagggccacggatttcatgtctc  
 ctgtga

GPI anchor

GPI anchor



**FIGURE 49B****Amino acid sequence of modified Env including multi-clade V3 loops [SEQ ID NO: 33]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | T | E | K | L | W | V |
| A | V | Y | Y | G | V | P | V | W | K | E | A | T | T | T | L | F | C |
| A | S | D | A | K | A | P | D | T | E | V | H | N | V | W | A | T | H |
| E | C | V | P | T | D | W | N | P | Q | E | V | V | L | V | N | V | T |
| I | N | F | N | M | Q | K | N | D | M | V | V | Q | M | H | E | D | I |
| V | S | A | W | S | C | S | L | S | P | C | T | A | L | T | P | L | C |
| S | K | E | N | I | P | I | T | Y | V | A | P | C | G | C | A | K | V |
| K | V | Q | T | H | G | E | H | P | V | R | S | T | Q | N | V | I | L |
| G | A | S | A | E | V | E | L | V | I | S | V | A | N | F | L | S | T |
| A | P | K | I | I | R | Q | S | N | Q | R | Q | E | I | N | T | D | N |
| P | F | N | N | T | K | I | G | K | M | V | R | A | H | C | G | T | R |
| T | F | P | T | N | D | I | R | S | D | I | R | Q | A | P | C | Q | A |
| F | R | A | N | G | T | Q | I | G | I | R | I | G | H | G | C | C | T |
| R | Y | T | N | E | I | Q | R | D | P | A | Q | L | C | Q | C | A | F |
| Y | P | N | R | I | R | D | I | R | R | I | G | C | V | T | A | T | R |
| P | T | T | R | T | E | I | R | I | G | A | H | Q | C | F | R | Y | S |
| T | G | I | T | G | S | I | R | K | A | P | G | Q | S | C | T | P | T |
| G | N | N | I | R | K | D | I | S | L | Y | C | G | R | V | R | N | T |
| A | N | E | I | K | S | I | R | F | K | A | H | Q | C | L | A | S | R |
| N | G | T | N | N | D | L | K | Q | A | D | S | L | G | N | E | Q | F |
| G | A | I | K | T | I | C | F | G | I | S | F | Y | C | R | P | E | I |
| V | L | G | S | F | N | F | N | S | T | W | S | T | K | D | S | T | N |
| L | T | Q | S | D | T | I | T | L | P | C | R | I | K | G | I | I | N |
| M | R | Q | S | V | G | K | A | M | L | A | P | P | I | S | G | Q | I |
| R | N | S | S | N | I | T | R | V | P | G | T | R | D | G | G | N | S |
| R | P | E | L | E | K | Y | K | V | V | K | I | E | M | L | G | V | A |
| L | T | L | A | K | L | R | A | A | Q | S | T | A | V | A | Q | S | A |
| T | N | L | R | Q | I | Q | A | Q | L | H | G | I | Q | Q | T | V | N |
| G | Q | L | Q | L | I | W | G | I | S | A | V | L | I | C | T | K | D |
| V | M | W | N | A | S | S | S | C | K | G | N | E | S | I | W | I | A |
| S | E | I | K | E | A | Q | N | Q | I | S | N | Y | Q | T | L | L | H |
| E | W | D | S | W | S | L | L | W | N | E | F | N | I | S | E | N | L |
| W | A | I | F | S | W | L | . | L | L | L | L | S | L | S | L | L | Q |

**FIGURE 50A****1. DNA sequence of p17/24 in natural form [SEQ ID NO: 34]:**

atgggtgcgagagcgtcagtat taagcgggggagaattagatcgatgggaaaaaattcgggttaaggccagg  
 gggaaagaaaaatatataaattaaaacatatagtatgggcaagcagggaagctagaacgattcgcagttaatc  
 ctggcctgttagaaacatcagaaggctgtagacaaatactgggacagctacaaccatcccttcagacagga  
 tcagaagaacttagatcattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaa  
 agacaccaaggaagctttagacaagatagaggaagagcaaaacaaaagtaagaaaaagcacagcaagcag  
 cagctgacacaggacacagcagtcaggtcagccaaaattaccctatagtgacagaacatccaggggcaaatg  
 gtacatcaggccatatcacctagaactttaaatgcatgggttaaaagtagtagaagagaaggctttcagccc  
 agaagtaatacccatgttttcagcattatcagaaggagccaccccacaagatttaaacaccatgctaaaca  
 cagtgggggggacatcaagcagccatgcaaatgtttaaagagaccatcaatgaggaagctgcagaatgggat  
 agagtacatccagtgcatgcagggcctattgcaccaggccagatgagagaaccaaggggaagtgcataagc  
 aggaactactagtagtacccttcaggaacaaataggatggatgacaaataatccacctatccagtaggagaaa  
 ttataaaagatggataatcctgggattaaataaaatagtaagaatgtatagccctaccagcattctgggac  
 ataagacaaggacaaaagaaccttttagagactatgtagaccggttctataaaactctaagagccgagca  
 agcttcacaggaggtaaaaaattggatgacagaaaaccttgttggtccaaaatgcgaaccagattgttaaga  
 ctatttttaaagcattgggaccagcggtacactagaagaaatgatgacagcatgtcagggagtaggagga  
 cccggccataaggcaagagttttgtaa

**2. DNA sequence of p17/24 in secreted form [SEQ ID NO: 35]:**

atgagagtgaaggagaaatatcaaccacttctggagatgggggtggagatcg  
 gp120 signal peptide  
ggcaccatgctccttgggatcttgatgatctgtagtgcctgggtgcgagagcg  
 p17/p24

tcagtattaaagcgggggagaattagatcgatgggaaaaaattcgggttaaggccagggggaaagaaaaata  
 taaattaaaacatatagtatgggcaagcagggaagctagaacgattcgcagttaatcctggcctgttagaaa  
 catcagaaggctgtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttaga  
 tcattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagacaccaaggaagc  
 ttttagacaagatagaggaagagcaaaacaaaagtaagaaaaaagcacagcaagcagcagctgacacaggac  
 acagcagtcaggtcagccaaaattaccctatagtgacagaacatccaggggcaaatggtacatcaggccata  
 tcacctagaacttttaaatgcatgggttaaaagtagtagaagagaaggctttcagcccagaagtaatacccat  
 gttttcagcattatcagaaggagccaccccacaagatttaaacacccatgctaaacacagtggtgggggacatc  
 aagcagccatgcaaatgtttaaagagaccatcaatgaggaagctgcagaatgggatagagtacatccagt  
 catgcagggcctattgcaccaggccagatgagagaaccaaggggaagtgcataagcaggaactactagtac  
 ccttcaggaacaaataggatggatgacaaataatccacctatccagtaggagaaatttataaaagatgga  
 taatcctgggattaaataaaatagtaagaatgtatagccctaccagcattctggacataagacaaggacca  
 aaagaaccttttagagactatgtagaccggttctataaaactctaagagccgagcaagcttcacaggaggt  
 aaaaaattggatgacagaaaccttgttggtccaaaatgcgaaccagattgttaagactatttttaaagcat  
 tgggaccagcggtacactagaagaaatgatgacagcatgtcagggagtaggaggaacccggccataaggca  
 agagttttgtaa

**FIGURE 50A -continued****1. DNA sequence of p17/24 in membrane form (SEQ ID NO: 36):**

atgagagtggaaggagaaatatcagcacttgcggaatcggggtggaagatgg

gp120 signal peptide

Ggcaccatgctccttgggatgttgatgatctctagtgcctgggtgcgagagcg

P17/p24

tcagttattaagcgggggagaattagatcgatgggaaaaaattcgggttaaggccagggggaaagaaaaata  
 taaattaaaacatatagtatgggcaagcaggagctagaacgattcgcagttaatcctggcctgttagaaa  
 catcagaaggctgtagacaaatactgggacagctacaaccatcccttcagacaggatcagaagaacttaga  
 tcattatataatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagacaccaaggaagc  
 tttagacaagatagaggaagagcaaaaacaaaagtaagaaaaaagcacagcaagcagcagctgacacaggac  
 acagcagtcaggtcagccaaaattaccctatagtgcaaacatccaggggcaaatggtacatcaggccata  
 tcacctagaactttaaatgcatgggtaaaagttagtagaagagaaggctttcagcccagaagtaatacccat  
 gttttcagcattatcagaaggagccaccccacaagattttaaacaccatgctaaacacagtggggggacatc  
 aagcagccatgcaaatgtttaaagagaccatcaatgaggaagctgcagaatgggtagagtacatccagtgc  
 catgcaggggcctattgcaccaggccagatgagagaaccaaggggaagtgcatagcaggaactactagtac  
 ccttcaggaacaaataggatggatgacaaataatccacctatcccagtaggagaaatttataaaagatgga  
 taatcctgggattaaataaaatagtaagaatgtatagccctaccagcattcttgacataagacaaggacca  
 aaagaaccttttagagactatgtagaccggttctataaaactctaagagccgagcaagcttcacaggaggt  
 aaaaaattggatgacagaaaccttgttggccaaaatgcgaaccagattgtaagactattttaaaagcat  
 tgggaccagcggctacactagaagaaatgatgacagcatgtcagggagtaggaggaccggccataaggca  
 agagttttg

ttattcataatgtagtaggagggcttggtaggtttaagaatagtttttgctgtactttctgtagtgaatag

agttagggcagggatattcaccattatcgtttcagacccacctcccaatcccgggggacaa

gp41 transmembrane domain

**FIGURE 50B****1. Amino acid sequence of p17/24 in natural form (SEQ ID NO: 37):**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | G | A | R | A | S | V | L | S | G | G | E | L | D | R | W | E | K |
| I | R | L | R | P | G | G | K | K | K | Y | K | L | K | H | I | V | W |
| A | S | R | E | L | E | R | F | A | V | N | P | G | L | L | E | T | S |
| E | G | C | R | Q | I | L | N | Q | L | Q | P | S | L | L | T | G | S |
| R | I | E | I | K | D | T | K | E | A | A | T | L | I | E | V | H | Q |
| N | K | S | K | K | K | A | Q | Q | A | A | D | K | T | G | H | S | S |
| Q | V | S | Q | N | Y | P | I | V | Q | N | I | Q | G | Q | M | V | H |
| A | A | I | S | P | R | T | L | N | A | W | V | K | V | V | E | A | K |
| P | Q | D | L | N | V | I | L | N | F | S | A | L | S | Q | A | A | T |
| Q | M | L | K | E | T | I | N | E | E | V | G | G | H | Q | A | V | M |
| P | V | H | A | G | P | I | A | P | Q | A | M | R | E | D | R | G | S |
| D | I | A | G | T | T | S | T | L | Q | E | Q | I | W | I | M | T | N |
| N | K | I | V | R | M | Y | S | P | Y | K | R | L | D | I | R | Q | L |
| P | K | E | P | F | R | D | Y | V | D | R | I | Y | K | T | L | R | A |
| E | Q | A | S | Q | E | V | K | N | W | M | T | E | T | L | L | V | Q |
| N | A | N | P | D | C | K | T | I | L | K | A | L | G | P | A | A | T |
| L | E | E | M | T | A | C | Q | G | V | G | G | G | P | G | H | K | A |
| R | V | L | * |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

**2. Amino acid sequence of p17/24 in secreted form (SEQ ID NO: 38):**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | G | A | R | A | S | V |
| L | S | G | G | E | L | D | R | W | E | K | I | R | R | R | P | G | G |
| K | K | K | Y | K | L | K | H | I | V | W | A | S | R | E | L | E | R |
| F | A | V | N | P | G | L | L | E | T | S | E | G | C | R | Q | I | L |
| G | Q | L | Q | P | S | L | Q | T | G | S | E | E | E | I | K | D | Y |
| N | T | V | A | T | L | Y | C | V | H | Q | R | I | E | I | K | K | A |
| K | E | A | A | D | K | I | G | E | S | S | Q | V | S | Q | N | K | P |
| Q | Q | A | A | D | D | T | G | H | S | S | Q | V | S | Q | N | Y | P |
| Q | Q | A | A | A | D | T | G | H | S | S | Q | V | S | Q | N | Y | P |
| I | V | Q | A | I | Q | G | V | Q | M | V | Q | A | I | S | P | R | T |
| L | N | A | F | V | K | V | S | E | G | A | P | Q | S | L | E | V | I |
| P | N | T | V | A | G | H | Q | A | A | M | Q | M | D | L | K | T | M |
| L | E | E | A | A | E | W | D | R | V | H | P | V | H | A | G | T | I |
| A | P | Q | Q | M | R | E | P | R | G | S | D | I | A | G | T | T | S |
| T | L | Q | E | Q | I | G | W | I | T | N | N | P | I | I | P | V | G |
| S | I | T | S | I | W | I | I | L | Q | G | N | K | E | I | V | M | Y |
| Y | P | D | R | F | L | K | T | L | R | A | P | Q | A | P | R | R | D |
| T | I | L | K | A | G | G | P | A | A | T | R | E | E | S | F | E | V |
| C | Q | G | V | G | G | P | G | H | K | A |   | V | L | * | M | T | A |

**FIGURE 50B-continued****1. Amino acid sequence of p17/24 in membrane bound form [SEQ ID NO: 39]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | G | A | R | A | S | V |
| L | S | G | G | E | L | D | R | W | E | K | I | R | L | R | P | G | G |
| L | S | G | G | E | L | D | R | W | E | K | I | R | L | R | P | G | G |
| K | K | K | Y | K | L | K | H | I | V | W | A | S | R | E | L | E | R |
| F | A | V | N | P | G | L | L | E | T | S | E | G | C | R | Q | I | L |
| G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | R | S | L | Y |
| N | T | V | A | T | L | Y | C | V | H | Q | N | I | E | I | K | D | T |
| K | E | A | L | D | K | I | E | E | S | Q | Q | K | S | K | K | K | A |
| Q | Q | A | A | A | D | T | G | H | S | S | Q | V | S | Q | N | Y | P |
| I | V | Q | N | I | Q | G | Q | M | V | H | Q | A | I | S | P | R | T |
| L | N | A | W | V | K | V | V | E | A | K | A | F | S | P | E | V | I |
| P | M | F | S | A | L | S | E | G | A | T | P | Q | D | L | N | T | M |
| L | N | T | V | G | G | H | Q | D | A | M | Q | V | L | K | E | T | I |
| N | E | E | A | A | E | W | D | R | V | H | P | V | H | A | G | P | S |
| A | P | G | Q | M | R | E | P | R | G | S | D | I | A | P | T | T | G |
| T | L | Q | E | Q | I | G | W | M | T | N | N | P | I | P | P | V | Y |
| E | I | Y | K | R | I | I | I | L | G | L | N | K | E | V | R | R | D |
| S | P | T | S | I | L | D | I | R | Q | A | P | K | A | P | F | R | V |
| Y | V | D | R | F | Y | K | T | L | R | Q | E | Q | A | S | Q | R | K |
| K | N | W | M | T | E | T | L | L | V | A | N | A | N | P | D | E | A |
| T | I | L | K | A | L | G | P | A | A | T | L | E | E | M | M | C | M |
| C | Q | G | V | G | G | P | G | H | K | A | R | V | L | L | F | I | V |
| I | V | G | G | Q | V | G | L | R | I | V | F | A | V | L | S | V | I |
| N | R | G | * | Q | G | Y | S | P | L | S | F | Q | T | H | L | P |   |

**FIGURE 51A****1. DNA sequence of p17 in natural form [SEQ ID NO: 40]:**

atgggtgcgagagcgctcagttattaagcgggggagaattagatcgatgggaaaaaattcg  
gttaaggccaggggaaagaaaaaatataaattaaaacatatagtatgggcaagcaggg  
agctagaacgattcgcagttaatcctggcctgttagaaacatcagaaggctgtagacaa  
atactgggacagctacaaccatcccttcagacaggatcagaagaacttagatcattata  
taatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagacaccaagg  
aagcttttagacaagatagaggaagagcaaaacaaaagtaagaaaaagcacagcaagca  
gcagctgacacaggacacagcagtcaggtcagccaaaattactaa

**2. DNA sequence of p17 in secreted form [SEQ ID NO: 41]:**

atgagagtgaaggagaaatatcagcacttctggagatgggggtggagatgg  
 gp120 signal peptide  
ggcaccatgctccttgggatggtgatgatctgtagtgcctggtgcgagagcg  
 p17  
tcagttattaagcgggggagaattagatcgatgggaaaaaattcgggttaaggccaggggg  
aaagaaaaaatataaattaaaacatatagtatgggcaagcagggagctagaacgattcg  
cagttaatcctggcctgttagaaacatcagaaggctgtagacaaatactgggacagcta  
caaccatcccttcagacaggatcagaagaacttagatcattatataatacagtagcaac  
cctctattgtgtgcatcaaaggatagagataaaagacaccaaggaagcttttagacaaga  
tagaggaagagcaaaacaaaagtaagaaaaaagcacagcaagcagcagctgacacagga  
cacagcagtcaggtcagccaaaattactaa

**3. DNA sequence of p17 in membrane bound form [SEQ ID NO: 42]:**

atgagagtgaaggagaaatatcagcacttctggagatgggggtggagatgg  
 gp120 signal peptide  
ggcaccatgctccttgggatggtgatgatctgtagtgcctggtgcgagagcg  
 p17  
tcagttattaagcgggggagaattagatcgatgggaaaaaattcgggttaaggccaggggg  
aaagaaaaaatataaattaaaacatatagtatgggcaagcagggagctagaacgattcg  
cagttaatcctggcctgttagaaacatcagaaggctgtagacaaatactgggacagcta  
caaccatcccttcagacaggatcagaagaacttagatcattatataatacagtagcaac  
cctctattgtgtgcatcaaaggatagagataaaagacaccaaggaagcttttagacaaga  
tagaggaagagcaaaacaaaagtaagaaaaaagcacagcaagcagcagctgacacagga  
cacagcagtcaggtcagccaaaattac  
ttattcataatgtagtaggagggcttggtaggtttaagaatagtttttgctgtactttc  
tgtagtgaatagagttagggcagggatattcaccattatcgtttcagacccacctccaa  
tcccgaaggggataa  
 gp41 transmembrane domain

FIGURE 51B**1. Amino acid sequence of p17 in natural form [SEQ ID NO: 43]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | G | A | R | A | S | V | L | S | G | G | E | L | D | R | W | E | K |
| I | R | L | R | P | G | G | K | K | K | Y | K | L | K | H | E | V | W |
| A | S | R | E | L | E | R | F | A | V | N | P | G | L | L | E | T | S |
| E | G | C | R | Q | I | L | G | Q | L | Q | P | S | L | Q | T | G | S |
| E | E | L | R | S | L | Y | N | T | V | A | T | L | Y | C | H | S | Q |
| R | I | E | I | K | D | T | K | E | A | L | D | K | I | E | S | Q | S |
| N | K | S | K | K | K | A | Q | Q | A | A | A | D | T | G |   |   |   |
| Q | V | S | Q | N | Y | * |   |   |   |   |   |   |   |   |   |   |   |

**2. Amino acid sequence of p17 in secreted form [SEQ ID NO: 44]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | G | A | R | A | S | V |
| L | S | G | G | E | L | D | R | W | E | K | I | R | L | R | P | G | G |
| K | K | K | Y | K | L | K | H | I | V | W | A | S | R | E | L | E | R |
| F | A | V | N | P | G | L | L | E | T | S | E | G | C | R | Q | I | L |
| G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | R | S | L | Y |
| G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | R | S | L | Y |
| N | T | V | A | T | L | Y | C | V | H | Q | R | I | E | I | K | D | T |
| K | E | A | L | D | K | I | E | E | E | Q | N | K | S | K | K | K | A |
| Q | Q | A | A | A | D | T | G | H | S | S | Q | V | S | Q | N | Y | * |

**3. Amino acid sequence of p17 in membrane bound form [SEQ ID NO: 45]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | G | A | R | A | S | V |
| L | S | G | G | E | L | D | R | W | E | K | I | R | L | R | P | G | G |
| K | K | K | Y | K | L | K | H | I | V | W | A | S | R | E | L | E | R |
| G | Q | L | Q | P | S | L | Q | T | G | S | E | E | L | R | S | L | Y |
| N | T | V | A | T | L | Y | C | V | H | Q | R | I | E | I | K | D | T |
| K | E | A | L | D | K | I | E | E | E | Q | N | K | S | K | K | K | A |
| Q | Q | A | A | A | D | T | G | H | S | S | Q | V | S | Q | N | Y | L |
| F | I | M | I | V | G | R | L | V | G | L | R | I | V | F | A | V | L |
| S | V | V | N | R | V | G | Q | G | Y | S | P | L | S | F | Q | T | H |
| L | P | I | P | R | G | * |   |   |   |   |   |   |   |   |   |   |   |

**FIGURE 52A****1. DNA sequence of p24 in natural form [SEQ ID NO: 46]:**

atgcctatagtgcagaacatccaggggcaaatsgtacatcagggccatatcacctagaactttaaatgcatgg  
gtaaaagttagtagaagagaaggctttcagcccagaagtaatacccatgttttcagcattatcagaaggagcc  
acccacaaagatttaaacaccatgctaaacacagtggggggacatcaagcagccatgcaaatgttaaaagag  
accatcaatgaggaagctgcagaatgggtagagtacatccagtgcatgcagggcctattgcaccaggccag  
atgagagaaccaaggggaagtgcatagcaggaactactagtacccttcaggaacaaataggatggatgaca  
aaatccacccatcccagtaggagaaattataaaagatggataatc  
ctgggattaaaataaaatagtaagaatgtatagccctaccagcattctggacataagacaaggacaaaagaa  
cccttttagagactatgtagaccgggtctataaaactctaagagccgagcaagcttcacaggaggttaaaaaat  
tggatgacagaaaccttctgtggtccaaaatgcgaaccagattgtaagactattttaaaagcattgggacca  
gcggctacactagaagaaatgatgcagcatgtcagggagtaggaggaccggccataaggcaagagtcttg  
taa

**2. DNA sequence of p24 in secreted form [SEQ ID NO: 47]:**

atgagagtgaaggagaaatatacagcactttatcgaagatgggggtggaagatgg  
 gp120 signal peptide ggcaccatgctccttgggatgttgatgatctgtagtctcctatagtgcag  
 p24  
aacatccaggggcaaatgggtacatcagggccatatcacctagaactttaaatgcatgggtaaaagttagtagaa  
gagaaggctttcagcccagaagtaatacccatgttttcagcattatcagaaggagccacccacaaagattta  
aacaccatgctaaacacagtggggggacatcaagcagccatgcaaatgttaaaagagaccatcaatgaggaa  
gctgcagaatgggtagagtacatccagtgcatgcagggcctattgcaccaggccagatgagagaaccaagg  
ggaagtgcatagcaggaactactagtacccttcaggaacaaataggatggatgacaaataatccacccatc  
ccagtaggagaaattataaaagatggataatcctgggattaaaataaaatagtaagaatgtatagccctacc  
agcattctggacataagacaaggacaaaagaaccttttagagactatgtagaccgggtctataaaactcta  
agagccgagcaagcttcacaggaggttaaaaaattggatgacagaaaccttctgtggtccaaaatgcgaacc  
gattgtaagactattttaaaagcattgggaccagcggtacactagaagaaatgatgcagcatgtcaggga  
gtaggaggacccggccataaggcaagagtcttgtaa

**3. DNA sequence of p24 in membrane bound form [SEQ ID NO: 48]:**

atgagagtgaaggagaaatatacagcactttatcgaagatgggggtggaagatgg  
 gp120 signal peptide ggcaccatgctccttgggatgttgatgatctgtagtctcctatagtgcag  
 p24  
aacatccaggggcaaatgggtacatcagggccatatcacctagaactttaaatgcatgggtaaaagttagtagaa  
gagaaggctttcagcccagaagtaatacccatgttttcagcattatcagaaggagccacccacaaagattta  
aacaccatgctaaacacagtggggggacatcaagcagccatgcaaatgttaaaagagaccatcaatgaggaa  
gctgcagaatgggtagagtacatccagtgcatgcagggcctattgcaccaggccagatgagagaaccaagg  
ggaagtgcatagcaggaactactagtaccttcagggaacaaataggatggatgacaaataatccacccatc  
ccagtaggagaaattataaaagatggataatcctgggattaaaataaaatagtaagaatgtatagccctacc  
agcattctggacataagacaaggacaaaagaaccttttagagactatgtagaccgggtctataaaactcta  
agagccgagcaagcttcacaggaggttaaaaaattggatgacagaaaccttctgtggtccaaaatgcgaacc  
gattgtaagactattttaaaagcattgggaccagcggtacactagaagaaatgatgcagcatgtcaggga  
gtaggaggacccggccataaggcaagagtcttg  
ctattcataatgataagtaagagctctgtaggcttaagaataagctttctgctgtacctctctgtagtaataga  
gttagggcagggatattcaccattatcctttcagacccacctcccaatcccgaaggataa  
 gp41 transmembrane domain



**FIGURE 52B****1. Amino acid sequence of p24 in natural form [SEQ ID NO: 49]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | P | I | V | Q | N | I | Q | G | Q | M | V | H | Q | A | I | S | S | P | E |
| R | T | L | N | A | W | V | K | V | V | E | E | K | A | F | S | D | L | K | N |
| V | I | P | M | F | S | A | L | S | E | A | A | T | P | Q | H | L | A | A | G |
| T | M | L | N | T | V | G | G | H | Q | A | V | M | Q | P | A | G | I | V | P |
| T | I | N | E | E | A | A | R | W | D | R | G | S | P | D | P | I | E | A | N |
| T | S | A | P | Q | Q | Q | I | E | P | R | T | N | N | N | P | E | A | N | E |
| V | G | T | I | Y | K | R | W | I | W | L | G | L | G | A | Q | A | E | V |   |
| M | Y | S | P | T | R | I | L | I | I | R | Q | R | V | A | T |   |   |   |   |
| R | D | Y | V | D | M | F | Y | T | T | L | L | A | K |   |   |   |   |   |   |
| E | V | K | N | W | K | T | E | T | L | L | A |   |   |   |   |   |   |   |   |
| C | K | T | I | L | V | A | L | G | P | G | H |   |   |   |   |   |   |   |   |
| T | A | C | Q | G | V | G | G | P | G |   |   |   |   |   |   |   |   |   |   |

**2. Amino acid sequence of p24 in secreted form [SEQ ID NO: 50]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | P | I | V | N | N | I |
| Q | G | Q | M | V | H | Q | A | I | S | P | R | T | L | N | W | N | V |
| K | V | V | E | A | K | A | F | S | D | L | V | I | P | L | S | V | A |
| L | S | E | G | A | T | P | Q | D | L | K | T | M | N | A | E | Q | E |
| G | H | Q | A | V | M | Q | M | H | A | A | P | I | S | T | G | K | S |
| E | R | D | R | G | S | D | I | P | I | G | V | S | Y | I | Y | R | M |
| R | I | P | M | T | N | N | P | K | I | P | R | D | K | T | D | W | L |
| I | W | I | L | Q | L | P | Q | E | A | N | E | V | K | I | G | V | G |
| W | L | I | R | R | G | N | A | N | E | L |   |   |   |   |   |   |   |
| L | Y | T | L | V | Q | E | N | E |   |   |   |   |   |   |   |   |   |
| E | T | L | L | A | T | L | E |   |   |   |   |   |   |   |   |   |   |
| L | G | P | A | K | A | R | V | L |   |   |   |   |   |   |   |   |   |
| G | P | G | H | K | A |   |   |   |   |   |   |   |   |   |   |   |   |

**3. Amino acid sequence of p24 in secreted form [SEQ ID NO: 51]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | R | V | K | E | K | Y | Q | H | L | W | R | W | G | W | R | W | G |
| T | M | L | L | G | M | L | M | I | C | S | A | P | I | V | N | N | I |
| Q | G | Q | M | V | H | Q | A | I | S | P | R | T | L | N | W | N | V |
| K | V | V | E | A | K | A | F | S | D | L | V | I | P | L | S | V | A |
| L | S | E | G | A | T | P | Q | D | L | K | T | M | N | A | E | Q | E |
| G | H | Q | A | V | M | Q | M | H | A | A | P | I | S | T | G | K | S |
| E | R | D | R | G | S | D | I | P | I | G | V | S | Y | I | Y | R | M |
| R | I | P | M | T | N | N | P | K | I | P | R | D | K | T | D | W | L |
| I | W | I | L | Q | L | P | Q | E | A | N | E | V | K | I | G | V | G |
| W | L | I | R | R | G | N | A | N | E | L |   |   |   |   |   |   |   |
| L | Y | T | L | V | Q | E | N | E |   |   |   |   |   |   |   |   |   |
| E | T | L | L | A | T | L | E |   |   |   |   |   |   |   |   |   |   |
| L | G | P | A | K | A | R | V | L |   |   |   |   |   |   |   |   |   |
| G | P | G | H | K | A |   |   |   |   |   |   |   |   |   |   |   |   |
| V | G | L | R | I | S | F | Q | T | H | L | P | I | P | R | G |   |   |
| G | Y | S | P | L | S |   |   |   |   |   |   |   |   |   |   |   |   |

**FIGURE 53A**

DNA sequence of modified Env including multi-clade V3 loops and Tat  
(SEQ ID NO: 52):

Gaattctgcaacaactgctgtttatccattttcagaattgggtgtcgacatagcagaataggcgt  
tactcgacagaggagagcaagaaatggagccagtagatcctagactagagccc

**Tat1**

Tggaagcatccaggaagtcagcctaaaaactgcttgtaccaattgctattgtaaaaagtgttgctt  
tcattgccaagtttgtttcatacaaaaagccttaggcattcctctatggcaggaagaagcggagac  
agcgacgaagacccctcaaggcagtcagactcatcaagtttctctatcaaagcagtagaagtagta  
catgtaatgcaacctatacaaatagcaatagtagcatttagtagtagcaataataatagcaatagt  
tgtgtggtccatagtaatcatagaatataggaaaaatattaagacaaaagaaaaatagacagggttaa  
ttgatagactaatagaaagagcagaagacagtggtgcaatgagagtgaaaggagaaatatcagcactt  
gtggagatgggggtggagatggg

**Envelope**

Gcaccatgctccttgggatgttgatgatctgtagtgtctacagaaaaattgtgggtcacagtcctat  
tatggggtacctgtgtggaaggaagcaaccaccactctattttgtgcatcagatgctaaagcata  
tgatacagaggtacataatgtttgggccacacatgctgtgtacccacagaccccaaccacaaag  
aagtagtatttgtaaatgtgacagaaaaattttaacatgtggaaaaatgacatggtagaacagatg  
catgaggatataatcagtttatgggatcaaagcctaaagccatgtgtgtaaaattaaccccactctg  
tgttggagctggttagttgtaacacctca

Delete V1V2, insert Gly,Ala,Gly

gtcattacacaggcctgtccaaagggtatcctttgagccaattcccatacattattgtgccccggc  
tggttttgcgattctaaaaatgtaataataagacgttcaatggaaacaggaccatgtacaaatgtca  
gcacagtagcaatgtacacatgggaattaggccagtagtatcaactcaactgctgttaaatggcagt  
ctggcagaagaagaggttagtaattagatctgccaatttcacagacaatgctaaaaccataatagt  
acagctgaaccaatctgtagaataattgtacaag

**First multi-clade repeat**

Acccaacaacaatacaagaaaaagtatccgtatccagagaggaccagggagagcatttgttaciaa  
taggaaaaataggaaatatgagacaagcacattgtctcgggtgtaccagacctaaacaataca  
agaaaaagtgtacgtataggaccaggacaaacatttctatgcaacaggtgatataataggggatat  
aagacaagcacattgttgtacgagacccaacaataatacaagaaaaagtataaggataggaccag  
gacaagcatttctatgcaacaggagaaataataggagatataagacaagcacattgttgcacaag  
ccctacaacaataataagacaagggacccccataggactagggcaagcactctatacaacaagaag  
aatagaagatataagaagagcacattgttgtaccagacctccaccantacaagaacaagtatac  
gtataggaccaggacaagtatttctatagaacaggagacataacaggagatataagaaaagcatat  
tgtggatcctgtacaagacccaacaacaatacaagaaaaagaatatctttaggaccaggacgagt  
atthttatacagcaggagaaataataggagacatcagaaaggcacattgttgtaccagacctata  
acaatacaagaaaaagtataacttttgcaccaggacaagcgctctatgcaacaggtgaaataata  
ggagatataagacaagcacattgtctcgggtgtaccagacctaaacaacaata

**Second multi-clade repeat**

Caagaaaaagtgtacgtataggaccaggacaaacatttctatgcaacaggtgatataataggggat  
ataagacaagcacattgttgtacgagacccaacaataatacaagaaagtataaggataggacc  
aggacaagcatttctatgcaacaggagaaataataggagatataagacaagcacattgttgcacaa  
ggccctacaacaataataagacaagggacccccataggactagggcaagcactctatacaacaaga  
agaatagaagatataagaagagcacattgttgtaccagacctccaccaatacaagaacaagtat  
acgtataggaccaggacaagtatttctatagaacaggagacataacaggagatataagaaaagcat  
atttgtggatcctgtacaagacccaacaacaatacaagaaaaagaatatctttaggaccaggacga  
gtattttatacagcaggagaaataataggagacatcagaaaggcacattgttgtaccagacctaa  
taacaatacaagaaaaagtataacttttgcaccaggacaagcgctctatgcaacaggtgaaataa

**FIGURE 53A-continued**

taggagatataagacaagcacattgctctgggaaacattagtagagcaaaatggaataacacttt

AvaI site, end of two multi-clade repeat

Aaaacagatagatagcaaattaagagaacaatttggaaataataaaacaataactcttaagcagt  
 cctcaggaggggacccagaaattgtaacgcagagttttaattgtggagggggaatttttctactgt  
 aattcaacacaactgttttaatagtacttgggttaatagtacttggagtactaaagggtcaaataa  
 cactgaagggaagtgcacacaatcacccctcccatgcagaataaaaacaaattataaacatgtggcagg  
 aagtaggaaaagcaatgtatgccccctcccatcagtggaacaaattagatgttcacaaatattaca  
 gggctgctattaaacaagagatggtggttaatagcaacaatgagtcagagatcttcagacctggagg  
 aggagatatgaggggacaattggagaagtgaattatataaatataaagtagtaaaaattgaaccat  
 taggagtagcaccacccaaggcaagagaagagtggtgcagactagtcagtgagggaataggagct  
 ttgttccttgg

Delete the cleavage site, insert SpeI site

gttcttgggagcagcaggaagcactatgggcgagcgtcaatgacgctgacggtacaggccagac  
 aattattgtctggtatagtgcagcagcagaacaatttgcctgagggctattgaggcgcaacagcat  
 ctgttgcaactcacagctctggggcatcaagcagctccaggcaagaatcctggctgtggaagata  
 cctaaaggatcaacagctcctggggatttgggggttgcctctggaaaactcatttgcaccactgctg  
 tgccttgggaatgctagttggagtaataaatctctggaacagatttgggaataacatgacctggatg  
 gagtgggacagagaaattaacaattacacaagcttaatacactccttaattgaagaatcgcaaaa  
 ccagcaagaaaagaatgaacaagaattattggaattagataaatgggcaagtttgtggaattggt  
 ttaacataacaaattggctgtggtatataaaattattcataatgatagtaggaggtctggtaggt  
 ttaagaatagtttttgcctgtactttctgtagtgaatagagttaggcagggatattcaccattatc  
 gtttcagacccacctcccaatcccagggggacccgacaggcccgaagggaatagaagaagaaggtg  
 gagagagagacagagacagatccattcgattagtgaaacggatccttagcacttatctggtaa

gp41, delete the 300 bp at C-terminal

**FIGURE 53B**

**Amino acid sequence of modified Env including multi-clade V3 loops and Tat  
[SEQ ID NO: 53]:**

```

M  R  V  K  E  K  Y  Q  H  L  W  R  W  S  W  R  L  W  G
T  M  L  L  G  M  L  P  I  C  S  A  T  T  E  T  N  F  V  C
T  V  Y  L  G  V  A  P  Y  W  T  E  V  H  N  V  L  W  F  T  H
A  S  D  A  P  K  T  D  W  Q  S  N  D  P  Q  M  L  A  N  E  D  I
E  N  F  N  M  D  W  K  S  N  L  N  V  Q  K  L  A  N  E  P  P  K
I  S  A  G  P  I  C  P  T  H  N  R  Y  C  I  A  G  T  C  F  N  L  L  T
V  G  C  C  N  T  K  E  V  R  G  P  V  I  Q  R  S  V  P  S  T  A  E  R  A
S  Q  S  L  T  A  I  T  G  E  V  L  S  I  R  S  V  Q  R  A  I  G  H  G  C
A  K  N  N  I  T  K  I  R  K  I  N  M  S  D  I  P  R  I  Q  G  A  L  C  Q
P  V  T  I  N  G  N  D  T  I  R  I  K  G  S  D  I  P  R  I  Q  G  A  L  C  Q
F  R  Y  P  A  N  T  G  N  E  I  R  E  S  D  I  R  I  K  G  S  D  I  R  E
T  R  Y  P  A  N  T  G  N  E  I  R  E  S  D  I  R  I  K  G  S  D  I  R  E
P  Y  T  N  T  I  T  G  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
T  T  N  D  I  T  T  G  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
G  N  A  N  G  N  E  T  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
N  G  N  E  N  D  T  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
T  G  N  E  I  R  E  S  D  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
R  R  I  T  T  G  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  I  K  G  S
T  T  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
I  I  K  G  S  D  I  R  I  K  G  S  D  I  R  I  K  G  S  D  I  R  E
I  N  K  S  T  F  N  W  T  G  I  K  R  V  V  Q  G  S  S  H  A  G  L  Q  L
S  S  T  D  V  N  E  Y  K  R  F  L  A  Q  A  R  G  S  N  E  Q  W  I  P  R
S  E  N  E  Y  K  R  F  L  A  Q  A  R  G  S  N  E  Q  W  I  P  R
S  L  A  G  V  R  Q  L  N  H  E  K  T  G  L

```

**FIGURE 54A**

DNA sequence of modified Env including multi-clade V3 loops, Tat and Rev  
[SEQ ID NO: 54]:

gaattctgcaacaactgctgtttatccattttcagaattgggtgtcgacatagcagaat  
aggcgttactcgacagaggagagcaagaaatggagccagtagatcctagactagagccc  
Tat1

tggaagcatccaggaagtcagcctaaaactgcttgtaccaattgctattgtaaaaagt  
ttgctttcattgccagtttgtttcatacaaaaagccttaggcatctcctatggcagga  
Rev1

agaagcggagacagcgacgaagacctcctcaaggcagtcagactcatcaagtttctcta  
tcaaagcagtaagtagtacatgtaatgcaacctatacaaatagcaatagtagcattagt  
agtagcaataataatagcaatagttgtgtgggtccatagtaatcatagaatatagggaaa  
tattaagacaaaagaaaaatagacaggttaattgatagactaatagaaagagcagaagac  
agtggcaamtgagagtggaaggagaaatatcagcacttgtggagatgggggtggagatggg  
Envelope

Gcaccatgctccttgggatgttgatgatctgtagtgtctacagaaaaattgtgggtcaca  
gtctattatggggtacctgtgtggaaggaagcaaccaccactctattttgtgcatcaga  
tgctaaagcatatgatacagaggtacataatgtttgggccacacatgcctgtgtaccca  
cagaccccaacccacaagaagtagtattggtaaatgtgacagaaaaattttaacatgtgg  
aaaaatgacatggtagaacagatgcatgaggatataatcagtttatgggatcaaagcct  
aaagccatgtgtaaaaattaacccactctgtgttggagctggtagttgtaacacctca  
Delete V1V2, insert Gly, ala, gly

gtcattacacaggcctgtccaaaggtatcctttgagccaattcccatacattattgtgc  
cccggctgggttttgcgattctaaaaatgtaataataagacggttcaatggaacaggacat  
gtacaaatgtcagcacagtacaatgtacacatggaattaggccagtagtatcaactcaa  
ctgctgttaaatggcagtcctggcagaagaagaggtagtaattagatctgccaatttcac  
agacaatgctaaaaccataatagtagcagctgaaccaatctgtagaattaattgtacaa  
g

## First multi-clades repeat

Acccaacaacaataacaagaaaaagtatccgtatccagagaggaccagggagagcatttg  
ttacaataggaaaaataggaaatatgagacaagcacattgtctcgggtgtaccagacct  
aacaacaatacaagaaaaagtgtagtataggaccaggacaaacattctatgcaacagg  
tgatataataggggatataagacaagcacattgttgtacgagacccaacaataatacaa  
gaaaaagttataaggataggaccaggacaagcattctatgcaacaggagaaataatagga  
gatataagacaagcacattgttgcacaaggccctacaacaataataagacaaggagcccc  
cataggactagggcaagcactctatacaacaagaagaatagaagatataagaagagcac  
attgttgtaccagaccctccaccaataacaagaacaagtatacgtataggaccaggacaa  
gtattctatagaacaggagacataacaggagatatagaagaaagcatattgtggatcctg  
tacaagacccaacaacaatacaagaaaaagaatatctttaggaccaggacgagtatattt  
atacagcaggagaaataataggagacatcagaaaggcacattgttgtaccagacctaat  
aacaatacaagaaaaagttataacttttgcaccaggacaagcgtctatgcaacagggtga  
aataataggagatatagaacaagcacattgtctcgggtgtaccagacctaacacaata

## Second multi-clade repeat

caagaaaaagtgtagtataggaccaggacaaacattctatgcaacagggtgatataata  
ggggatatagaacaagcacattgttgtacgagacccaacaataatacaagaaaaagtat

FIGURE 54A-continued

aaggataggaccaggacaagcattctatgcaacaggagaaaataataggagatataagac  
 aagcacattgttgacacaaggccctacaacaatataagacaaaggaccccataggacta  
 gggcaagcactctatacaacaagaagaatagaagatataagaagagcacattgtgtac  
 cagaccctccaccaatacaagaacaagtatacgtataggaccaggacaagtattctata  
 gaacaggagacataacaggagatataagaaaagcatattgtggatcctgtacaagaccc  
 aacaacaatatacaagaaaaagaatatcttttaggaccaggacgagtattttatacagcagg  
 agaaataataggagacatcagaaaggcacattgttgtaccagacctaataacaatacaa  
 gaaaaagtataacttttgcaccaggacaagcgctctatgcaacagggtgaaataatagga  
 gatataagacaagcacattgtctcgggaacattagtagagcaaaatggaataacacttt

AvaI site, end of two multi-clade repeat

Aaaacagatagatagcaaatataagagaacaatttggaaataataaaacaataatcttta  
 agcagtcctcaggaggggacccagaaattgtaacgcacagttttaattgtggaggggaa  
 tttttctactgttaattcaacacaactgtttaatagtacttgggttaatagtacttggag  
 tactaaaggggtcaaataacactgaaggaagtgcacaatcacccctcccatgcagaataa  
 acaaatattataaacatgtggcaggaagttaggaaaagcaatgtatgcccctcccatcagt  
 ggacaaattagatgttcatcaaataattacagggctgctattaacaagagatggtggttaa  
 tagcaacaatgagtcagatccttcagacctggaggaggagatagaggggacaattgga  
 gaagtgaattatataaatataaagtagtaaaaattgaaccattaggagtagcaccacc  
 aaggcaagagaagagtggtgcagactagtgcagtggaataggagctttgttccttgg

Delete the cleavage site, insert SpeI

gttcttgggagcagcaggaagcactatgggctgcacgtcaatgacgctgacggtacagg  
 ccagacaattattgtctgatatagtgcagcagcagaacaatttgcaggggctattgag  
 gcgcaacagcatctgttgcaactcacagctctggggcatcaaacagctccaggcaagaat  
 cctggctgtggaaagatacctaaggaatcaacagctcctggggatttgggggtgtctctg  
 gaaaactcatttgcaccactgctgtgccttgggaatgctagttaggagtaataaatctctg  
 gaacagatttggaaataacatgacctggatggagtgaggacagagaaattaacaattacac  
 aagcttaatacactccttaattgaagaatcgcaaaaccagcaagaaaagaatgaacaag  
 aattattggaattagataaatgggcaagtttgtggaattgggttaacatacaaaattgg  
 ctgtgggtatataaaattattcataatgatagtaggaggcttggtaggtttaagaatagt  
 ttttgcgtgactttctatagtgaatagagttaggcagggatattcaccattatcgtttc  
 agacccacctcccaatcccaggggacccgacaggcccgaaaggaatagaagaagaaggt  
 ggagagagagacagagacagatccattcgattagtgaacggatccttagcacttatctg  
 ggacgatctgcggagcctgtgcctcttcagctaccaccgcttgagagacttactcttga  
 ttgtaacgaggattgtggaacttctgggacgcaggggggtgggaagccctcaaatattgg  
 tggaaatctcctacagtattggagtcaggaactaaagaatagtgcgtgtaacttgctcaa  
 tggcacagccatagcagtagctgagtaa

gp41, but 99 bp truncation at C-terminus

**FIGURE 54B**

Amino acid sequence of modified Env including multi-clade V3 loops. Tat and Rev  
[SEQ ID NO: 55]:

```

M T T A A E I V S K V G A P F T F R Y P T T G N A N G N T N G N R R T T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
R M V S C N S G F C Q S K N V R Y P T T G N A N G N T N G N R R T T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
V L Y D V F L A E N C L T N T P A N T R R T T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
K L Y A P N W G P N T A I N N G N R R T T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
E G K T M D S I K H E I T G N G N R R T T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
K M V A D W Q C P T G E V R K N D T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
Y L P Y P K S N I F I E Q K I T I R I N K S S E S L A G V R Q L N M E K K V T G L D E N
Q M V D N N L T H N R V L S G R I K G S D T R I K S R F Q R R I Q G H G C P H G C S S F P T G I S T D L V K L N K F G R P S L I L A
H W T P D K S Y G P V N I N K G S D T R I K S R F Q R R I Q G H G C P H G C S S F P T G I S T D L V K L N K F G R P S L I L A
L C K E Q M P V C T V I Q R M S D I I P R G A L K A A I Q G A L C Q G C Q L K G Y T I P R D E A M I L E Y N N L Q D I C V L T
W S E V E V C I A G V R S I R V I R R I A P Y G A P H G A P H G C Q V S R C A G L G C K K I D M P V G V Q R I Q T E I V G R R L F L Y I
R A A H V E V T P P S S V Q Q R R I Q G H G C P H G C Q V S R C A G L G C K K I D M P V G V Q R I Q T E I V G R R L F L Y I
W T T N V Q K Q A C T A E R A I Q G A L C Q G C Q L K G Y T I P R D E A M I L E Y N N L Q D I C V L T
G E T V L M L A G T Q N I G H G A P H G C V S R C A G C C A T L P R R Y P A S Q E T N I Q N N V G S Q V K T N I L W R P G N Y G S V
W K T W V H T C F N L F N P C P H G C Q T F C V T L C T T F R Y S T P T N T G N A N G N E K N T F E W C N S T F L L I Q P T L Y F F E L L G L
R L L A N E P P A V L T C G L G C Q C A R R Y T F R Y P T T G N A N G N E K N T F E W C N S T F L L I Q P T L Y F F E L L G L
W F T V D L K I S L D T R G Q C A T L P R R Y P A R Y P A Y T N D N G N E K N T F E W C N S T F L L I Q P T L Y F F E L L G L
G V C H T I C V L T N N R A C T T F R Y S T P T N T P A N T N R T I N E T I W N H N G Q S E E K L T L K L W W I D I A Q E A R W K

```

**FIGURE 55A**

DNA sequence of HIV-1 (strain BH10) Protease (PI, nt 1407-1907) [SEQ ID NO: 56]:

atgttcttagggaagatctggccttctacaagggaaggccagggaattttctcagagcagaccagagcca  
acagccccaccatttctcagagcagaccagagccaacagccccaccagaagagagcttcagggtctggggt  
agagacaacaactccccctcagaagcaggagccgatagacaaggaaactgtatccttaacttccctcagatc  
actctttggcaacgacccctcgtcacaataaagataggggggcaactaaaggaagctctattagatacagga  
gcagatgatacagtattagaagaaatgagtttccagggaagatggaaacaaaaatgatagggggaattgg  
aggttttatcaaagtaagacagtatgatcagatactcatagaaatctgtggacataaagctataggtacagtatt  
agtaggacctacacctgtcaacataattggaagaaatctgttgactcagattggttcacittaaattttaa

**FIGURE 55B**

Amino acid sequence of HIV-1 (strain BH10) Protease (PI) [SEQ ID NO: 57]:

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | F | F | R | E | D | L | A | F | L | Q | G | K | A | R | E | F | S |
| S | E | Q | T | R | A | N | S | P | T | I | S | S | E | Q | T | R | A |
| N | S | P | T | R | R | E | L | Q | V | W | G | R | D | N | N | S | P |
| S | E | A | G | A | D | R | Q | G | T | V | S | F | N | F | P | Q | I |
| T | L | W | Q | R | P | L | V | T | I | K | I | G | G | Q | L | K | E |
| A | L | L | D | T | G | A | D | D | T | V | L | E | E | M | S | L | P |
| G | R | W | K | P | K | M | I | G | G | I | G | G | F | I | K | V | R |
| Q | Y | D | Q | I | L | I | E | I | C | G | H | K | A | I | G | T | V |
| L | V | G | P | T | P | V | N | I | I | G | R | N | L | L | T | Q | I |
| G | C | T | L | N | F | * |   |   |   |   |   |   |   |   |   |   |   |



**FIGURE 56A**

DNA sequence of HIV-1 (strain BH10) Gag-PI [SEQ ID NO: 58]:

Atgggtgcgagagcgtcagttattaagcgggggagaattagatcgatgggaaaaattcg  
gttaaggccaggggaaagaaaaatatataaattaaaacatatagtatgggcaagcagg  
agctagaacgattcgagtttaacctggcctgttagaaacatcagaaggctgtagacaa  
atactgggacagctacaaccatcccttcagacaggatcagaagaacttagatcattata  
taatacagtagcaaccctctattgtgtgcatcaaaggatagagataaaagacaccaagg  
aagcttttagacaagatagaggaagagcaaaaacaaaagtaagaaaaagcacagcaagca  
gcagctgacacaggacacagcagtcagggtcagccaaaattaccctatagtcagaacat  
ccaggggcaaatggtacatcaggccatatacctagaacttttaaatgcatgggtaaaag  
tagtagaagagaaggctttcagcccagaagtaatacccatgttttcagcattatcagaa  
ggagccacccccacaagatttaaacaccatgctaaacacagtggggggacatcaagcagc  
catgcaaatgtttaaagagaccatcaatgaggaagctgcagaatgggtagagtacatc  
cagtgcagtcaggggcctattgcaccaggccagatgagagaaccaaggggaagtgcata  
gcaggaactactagtacccttcaggaacaaataggatggatgacaaataatccacctat  
cccagtaggagaaaatttataaaagatggataatcctgggattaaataaaatagtaagaa  
tgtatagccctaccagcattctggacataagacaaggacccaaaagaaccttttagagac  
tatgtagaccggttctataaaaactctaagagccgagcaagcttcacaggagggtaaaaaa  
ttggatgacagaaaccttggtggtccaaaatgcgaaccagattgtaagactattttaa  
aagcattgggaccagcggctacactagaagaaatgatgacagcatgtcaggggagtagga  
ggacccggccataaggcaagagttttggctgaagcaatgagccaagtaacaaatacagc  
taccataatgatgcagagaggcaatttttaggaaccaagaaagatgggttaagtgtttca  
attgtggcaagaagggcacacagccagaaaattgcaggggccctaggaaaaagggtgt  
tggaatgtggaaaggaaggacaccaaatgaaagattgtactgagagacaggctaattt  
ctttagggaagatctggccttcctacaagggaaggccagggaattttcttcagagcaga  
ccagagccaacagccccaccatttcttcagagcagaccagagccaacagccccaccaga  
agagagcttcagggtctggggtagagacaacaactccccctcagaagcaggagccgatag  
acaaggaactgtatcctttaactccctcagatcactctttggcaacgacccctcgta  
caataaagataggggggcaactaaaggaagctctattagatacaggagcagatgataca  
gtattagaagaaatgagtttgccaggaagatggaaacccaaaatgataggggggaattgg  
aggttttatcaaagtaagacagtatgatcagatactcatagaaatctgtggacataaag  
ctataggtacagttattagtaggacctacacctgtcaacataattggaagaaatctgttg  
actcagattggttgcactttaaatttttaa

**FIGURE 56B****Amino acid sequence of HIV-1 (strain BH10) Gag-PI [SEQ ID NO: 59]:**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | G | A | R | A | S | V | L | S | G | G | E | L | D | R | W | E | K |
| I | R | L | R | P | G | G | K | K | E | R | F | L | K | H | I | V | W |
| A | S | R | E | L | E | R | K | A | I | K | Q | L | L | L | E | T | S |
| E | G | C | R | Q | I | L | F | Q | T | L | G | L | Y | Q | T | G | S |
| R | I | E | I | K | D | T | K | E | Q | A | A | K | I | E | H | E | Q |
| N | K | S | K | N | Y | P | Q | V | I | A | Q | D | T | G | M | S | S |
| Q | V | S | Q | P | R | T | I | N | M | F | A | K | V | Q | E | V | H |
| A | F | S | P | E | V | I | L | N | E | S | W | L | G | V | A | E | K |
| P | Q | D | L | N | T | M | P | N | T | V | A | G | S | E | G | A | T |
| Q | M | L | K | E | T | I | I | E | G | I | Q | E | H | Q | R | A | M |
| P | V | H | A | G | P | S | A | P | Q | Y | E | R | I | D | M | V | S |
| D | I | A | G | T | V | G | T | E | Q | T | K | R | L | I | L | G | A |
| N | K | I | V | R | M | Y | S | I | P | D | R | F | L | I | R | Q | N |
| P | K | E | P | F | R | D | Y | V | N | W | M | T | E | T | L | V | A |
| E | Q | A | S | Q | E | V | K | I | I | L | K | A | L | P | A | A | Q |
| N | A | N | P | D | C | K | T | Q | Q | G | V | G | T | G | H | K | T |
| L | E | L | M | E | T | A | C | S | R | V | T | N | T | P | I | M | A |
| Q | R | G | A | F | A | N | R | Q | C | K | R | V | K | T | N | C | M |
| K | E | G | H | T | A | R | H | N | M | D | K | A | R | K | G | C | W |
| K | C | G | K | E | G | A | S | Q | L | G | Q | C | E | R | Q | A | N |
| F | F | R | E | D | L | A | F | P | I | S | I | A | Q | N | F | S | S |
| E | Q | T | R | A | N | S | L | V | T | G | W | D | T | N | R | A | N |
| S | P | G | A | R | E | Q | Q | G | T | V | V | N | F | P | S | P | S |
| E | A | Q | R | D | R | V | T | I | I | K | I | G | Q | N | K | I | T |
| L | W | D | T | P | L | D | D | G | I | V | L | E | M | S | L | E | A |
| R | D | K | P | K | A | I | I | I | C | I | H | F | I | K | V | P | Q |
| Y | G | Q | I | L | M | E | I | I | I | G | R | A | L | G | T | R | L |
| V | T | P | N | P | I | N | I | I | I | G | N | L | L | T | I | V | G |
| C | T | L | N | F | * | N | I | I | I | G | R | L | L | T | Q | I | G |

**FIGURE 57**

**Primers for multi-clade V3 loops:**

Clade A: (1). forward primer A888F5 [SEQ ID NO: 60]:

5'-aaa tca acc gga att gaa ttc cct cgg gtg tac cag acc taa caa caa tac-3'

EcoRI    AvaI

(2). reverse primer A-CR3 [SEQ ID NO: 61]:

5'-att gtt ggg tct cgt aca aca atg tgc ttg tct tat atc ccc-3'

Clade C: (3). forward primer A-CF5 [SEQ ID NO: 62]:

5'-ggg gat ata aga caa gca cat tgt acg aga ccc aac aat ac-3'

(4). reverse primer C980R3 [SEQ ID NO: 63]:

5'-gtt gta ggg cct tgt gca aca atg tgc ttg tct tat atc-3'

**Clade D: (5). forward primer D888F5 [SEQ ID NO: 64]:**

5'-gat ata aga caa gca cat tgt tgc aca agg ccc tac aac-3'

(6). reverse primer D-ER3 [SEQ ID NO: 65]:

5'-ggg gga ggg tct ggt aca aca atg tgc tct tct tat-3'

**Clade E: (7). forward primer D-EF5 [SEQ ID NO: 66]:**

5' -ata aga aga gca cat tgt tgt acc aga ccc tcc acc-3'

(8). reverse primer E998R3 [SEQ ID NO: 67]:

5'-gta ttg ttg ttg ggt ctt gta caa caa tat gct ttt ctt ata tct cc-3'

**Clade F: (9). forward primer F888F5 [SEQ ID NO: 68]:**

5'-gga gat ata aga aaa gca tat tgt tgt aca aga ccc aac aac aat ac-3'

(10). reverse primer F-GR3 [SEQ ID NO: 69]:

5'-gtt att agg tct ggt aca aca atg tgc ctt tct gat gtc-3'

**Clade G: (11). forward primer F-GF5 [SEQ ID NO: 70]:**

5'-gac atc aga aag gca cat tgt tgt acc aga cct aat aac-3'

(12). reverse primer G989R3 [SEQ ID NO: 71]:

5'-aat aaa cta gtc tag acc ccc gag tct aga aca atg tgc ttg tct tat atc tcc-3'

AvaI XbaI

FIGURE 58

## pRAD-ORF6-Gag/PI-RT

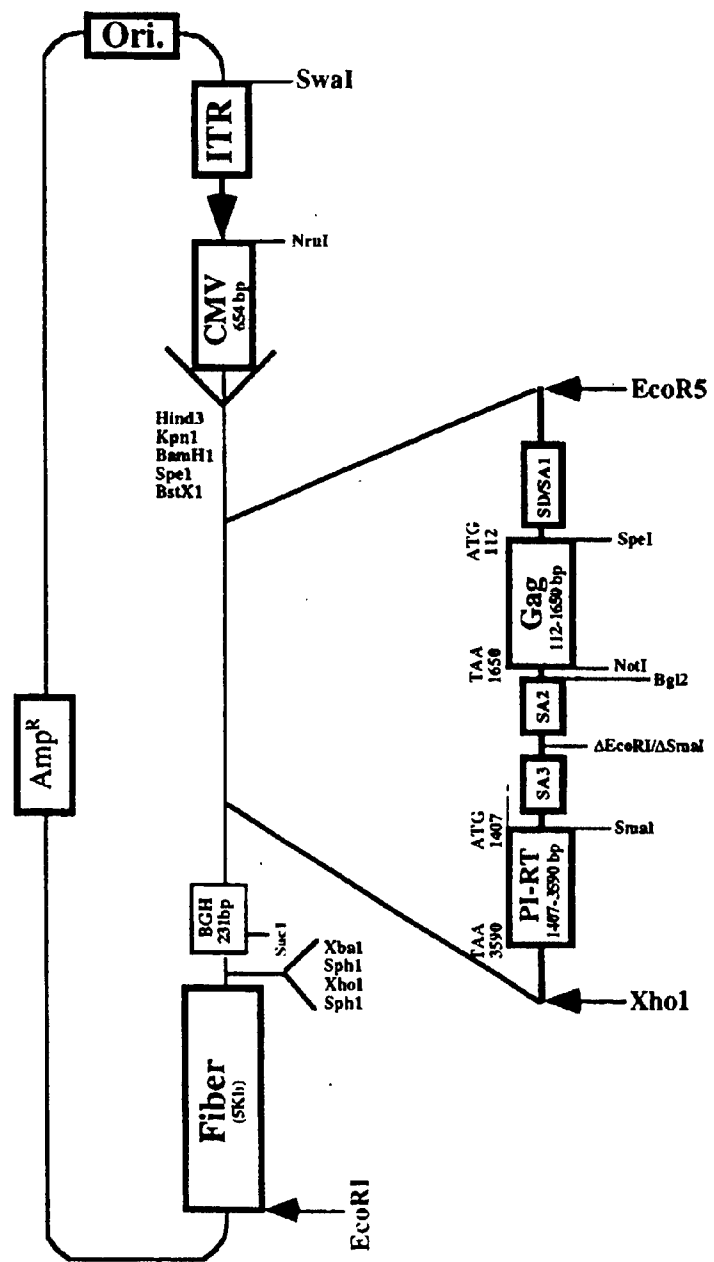


FIGURE S9

**pRad-ORF6-Gag-PI-RT**

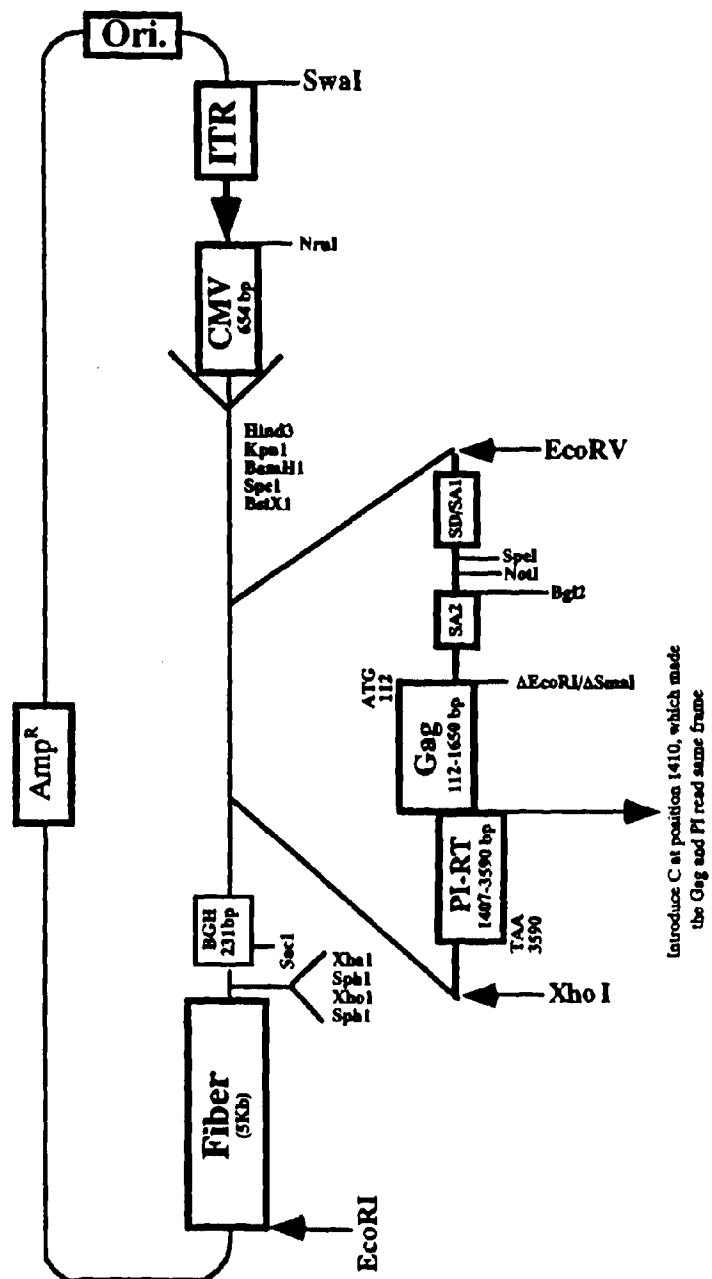


FIGURE 60

pRad-ORF6-Gag/Pol

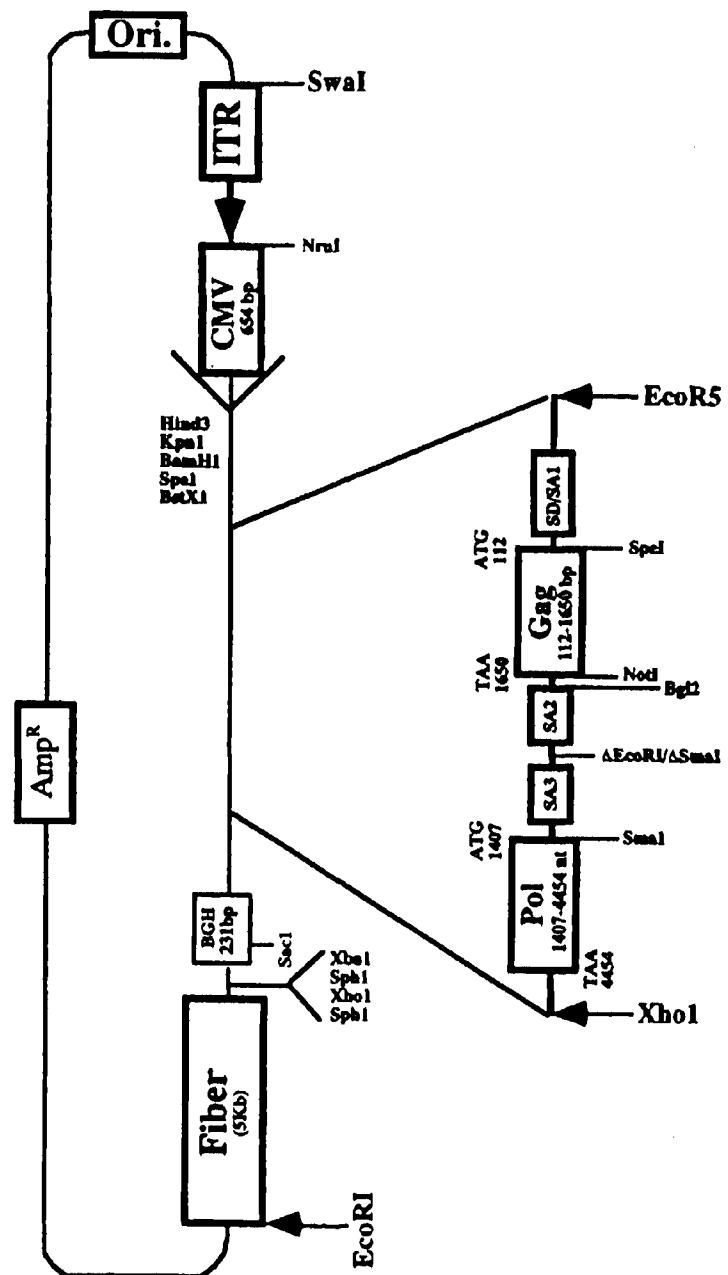
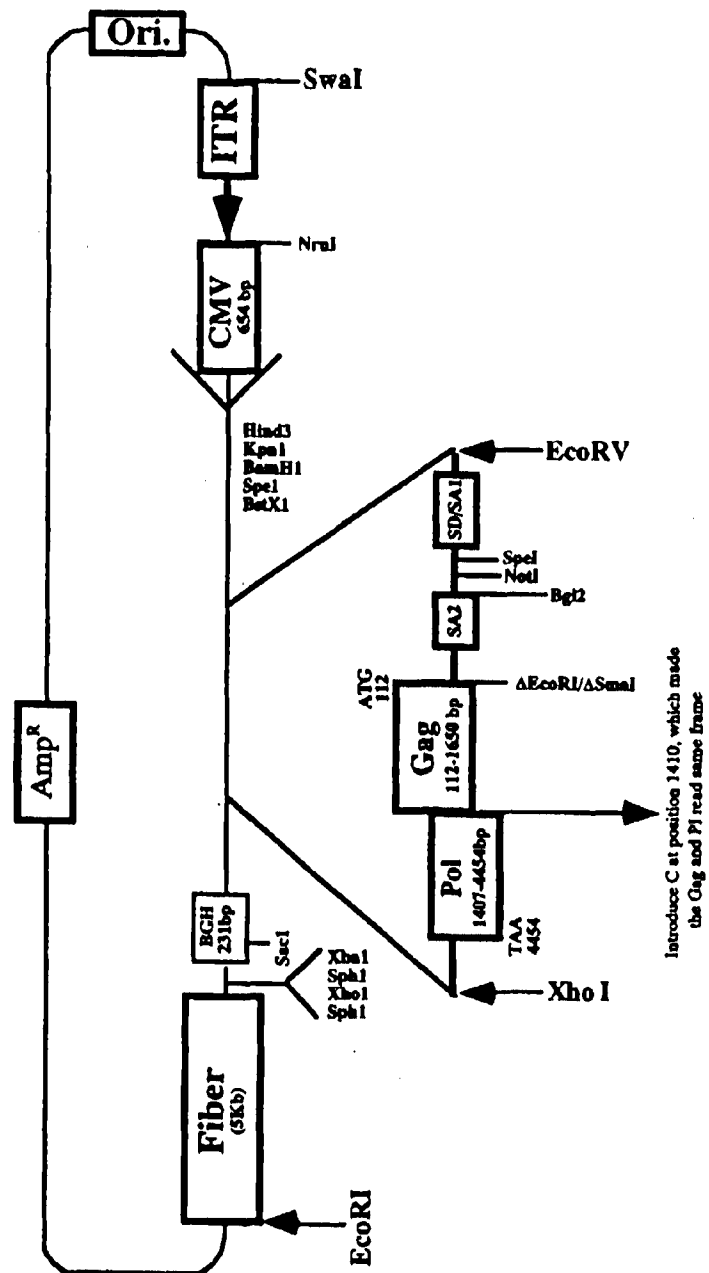
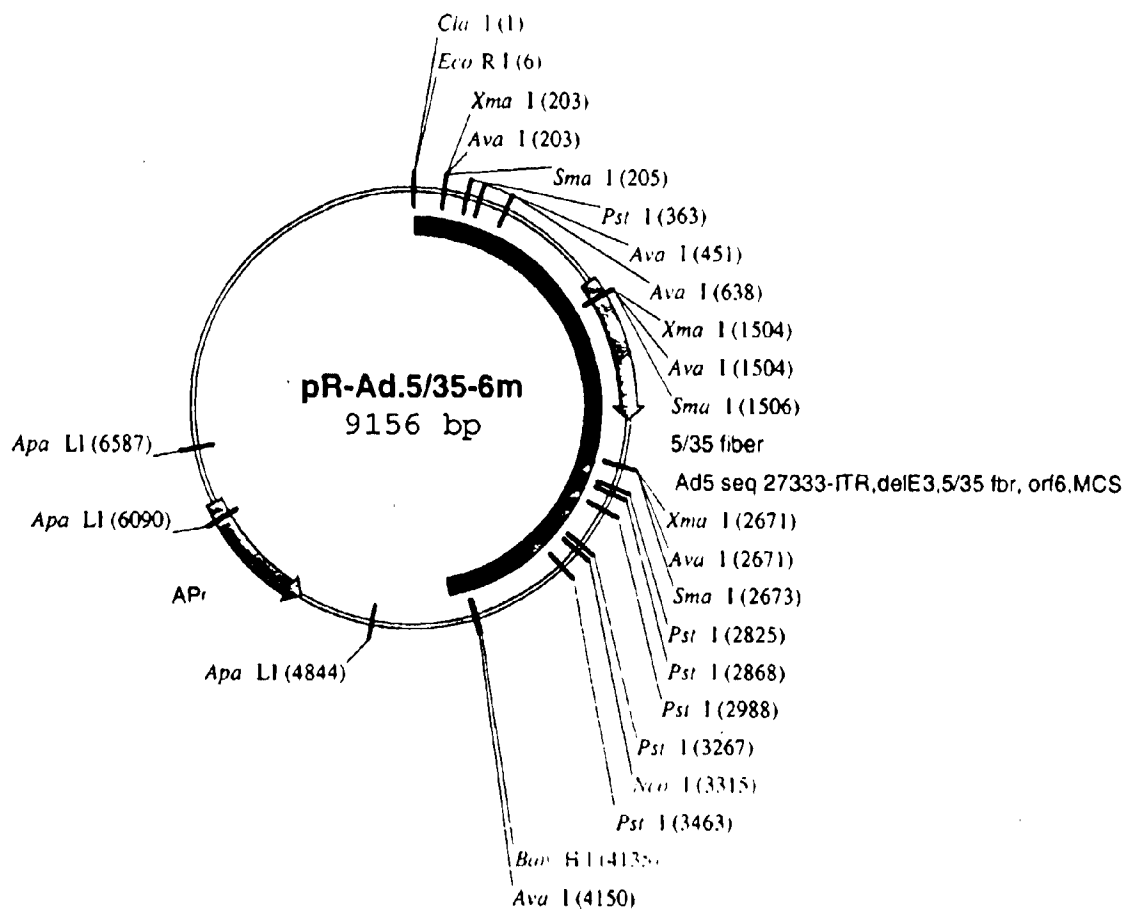
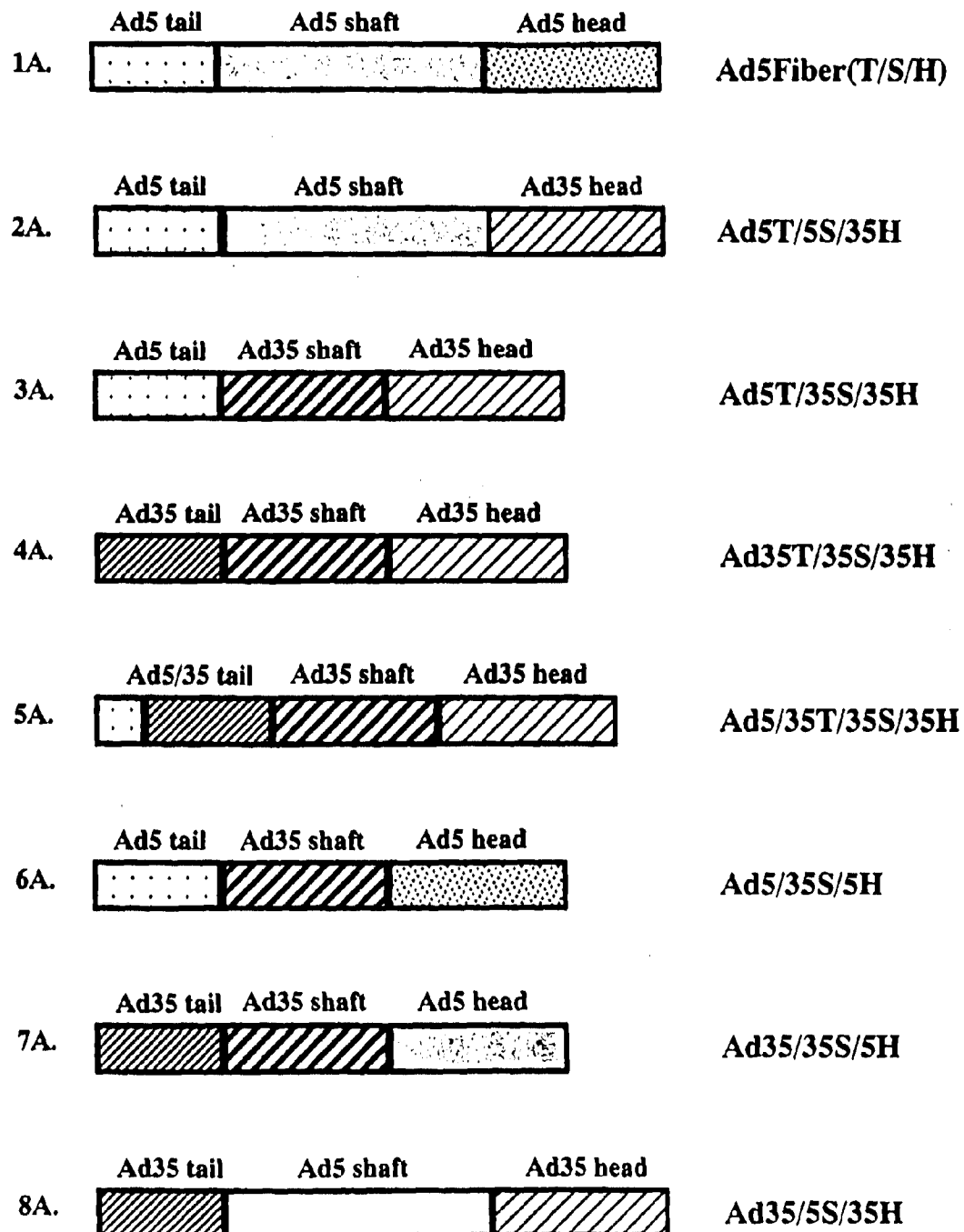


FIGURE 61  
pRad-ORF6-Gag-Pol



**FIGURE 62**



**FIGURE 63**

Genphar.740.ST25.txt  
SEQUENCE LISTING

&lt;110&gt; Wang, Danher

&lt;120&gt; GENETIC VACCINE AGAINST HUMAN IMMUNODEFICIENCY VIRUS

&lt;130&gt; 22488-740

&lt;150&gt; US 09/585,599

&lt;151&gt; 2000-06-02

&lt;150&gt; PCT/US01/18238

&lt;151&gt; 2001-06-04

&lt;150&gt; US 10/003,035

&lt;151&gt; 2001-11-01

&lt;160&gt; 75

&lt;170&gt; PatentIn version 3.1

&lt;210&gt; 1

&lt;211&gt; 7

&lt;212&gt; DNA

&lt;213&gt; Ebola virus

<400> 1  
ttttttt

7

&lt;210&gt; 2

&lt;211&gt; 7

&lt;212&gt; RNA

&lt;213&gt; Ebola virus

## Genphar.740.ST25.txt

<400> 2  
uuuuuuuu 7

<210> 3  
<211> 8  
<212> RNA  
<213> Artificial sequence

<220>  
<223> Modified RNA editing site

<400> 3  
uucuucuu 8

<210> 4  
<211> 7  
<212> DNA  
<213> Ebola virus

<400> 4  
aaaaaaa 7

<210> 5  
<211> 8  
<212> DNA  
<213> Artificial sequence

<220>  
<223> DNA of modified RNA editing site

<400> 5  
aagaagaa 8

<210> 6  
<211> 6  
<212> RNA

Genphar.740.ST25.txt

&lt;213&gt; Ebola virus

<400> 6  
uuuuuu

6

&lt;210&gt; 7

&lt;211&gt; 6

&lt;212&gt; RNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified RNA editing site

<400> 7  
uucuuc

6

&lt;210&gt; 8

&lt;211&gt; 6

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA of modified RNA editing site

<400> 8  
ttcttc

6

&lt;210&gt; 9

&lt;211&gt; 21

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

Ser Trp Leu Leu Leu Leu Leu Ser Leu Ser Leu Leu Gln Ala Thr  
1 5 10 15Asp Phe Met Ser Leu  
20

Genphar.740.ST25.txt

&lt;210&gt; 10

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Ebola virus

&lt;400&gt; 10

Arg Arg Thr Arg Arg  
1 5

&lt;210&gt; 11

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 11

Arg Glu Lys Arg  
1

&lt;210&gt; 12

&lt;211&gt; 9

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA of GAG site

<400> 12  
ggagctggt

9

&lt;210&gt; 13

&lt;211&gt; 3

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

## Genphar.740.ST25.txt

&lt;223&gt; GAG site

&lt;400&gt; 13

Gly Ala Gly

1

&lt;210&gt; 14

&lt;211&gt; 3157

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Env/Tat/Rev

&lt;400&gt; 14

|  |      |
|--|------|
| gaattctgca acaactgctg tttatccatt ttcagaattg ggtgtcgaca tagcagaata  | 60   |
| ggcgttactc gacagaggag agcaagaaat ggagccagta gatcctagac tagagccctg  | 120  |
| gaagcatcca ggaagtcagc ctaaaactgc ttgtaccaat tgctattgta aaaagtgttg  | 180  |
| ctttcattgc caagtttggt tcataacaaa agccttaggc atctcctatg gcaggaagaa  | 240  |
| gcggagacag cgacgaagac ctcctcaagg cagtcagact catcaagttt ctctatcaaa  | 300  |
| gcagtaagta gtacatgtaa tgcaacctat acaaatagca atagtagcat tagtagtagc  | 360  |
| aataataata gcaatagttg tgtggtccat agtaatcata gaatatagga aaatattaag  | 420  |
| acaaagaaaa atagacaggt taattgatag actaatagaa agagcagaag acagtggcaa  | 480  |
| tgagagtgaa ggagaaatat cagcacttgt ggagatgggg gtggagatgg ggcaccatgc  | 540  |
| tccttgggat gttgatgac tgtagtgcta cagaaaaatt gtgggtcaca gtctattatg   | 600  |
| gggtacctgt gtggaaggaa gcaaccacca ctctattttg tgcatcagat gctaaagcat  | 660  |
| atgatacaga ggtacataat gtttgggcca cacatgcctg tgtaccaca gacccaacc    | 720  |
| cacaagaagt agtattggta aatgtgacag aaaattttaa catgtggaaa aatgacatgg  | 780  |
| tagaacagat gcatgaggat ataatcagtt tatgggatca aagcctaaag ccatgtgtaa  | 840  |
| aattaacccc actctgtggt agtttaaagt gcactgattt gaagaatgat actaatacca  | 900  |
| atagtagtag cgggagaatg ataatggaga aaggagagat aaaaaactgc tctttcaata  | 960  |
| tcagcacaag cataagaggt aagggtgcaga aagaatatgc atttttttat aaacttgata | 1020 |
| taataccaat agataatgat actaccagct atacgttgac aagttgtaac acctcagtca  | 1080 |
| ttacacaggc ctgtccaaag gtatcctttg agccaattcc catacattat tgtgccccgg  | 1140 |
| ctggttttgc gattctaaaa tgtaataata agacgttcaa tggaacagga ccatgtacaa  | 1200 |

## Genphar.740.ST25.txt

|  |      |
|--|------|
| atgtcagcac agtacaatgt acacatggaa ttaggccagt agtatcaact caactgctgt  | 1260 |
| taaatggcag tctggcagaa gaagaggtag taattagatc tgccaatttc acagacaatg  | 1320 |
| ctaaaacat aatagtacag ctgaaccaat ctgtagaaat taattgtaca agaccaaca    | 1380 |
| acaatacaag aaaaagtatc cgtatccaga gaggaccagg gagagcattt gttacaatag  | 1440 |
| gaaaaatagg aaatatgaga caagcacatt gtaacattag tagagcaaaa tggaataaca  | 1500 |
| ctttaaaca gatagatagc aaattaagag aacaatttgg aaataataaa acaataatct   | 1560 |
| ttaagcagtc ctcaggaggg gaccagaaa ttgtaacgca cagttttaat tgtggagggg   | 1620 |
| aatttttcta ctgtaattca acacaactgt ttaatagtac ttggtttaat agtacttggg  | 1680 |
| gtactaaagg gtcaaataac actgaaggaa gtgacacaat caccctcca tgcagaataa   | 1740 |
| aacaaattat aaacatgtgg caggaagtag gaaaagcaat gtatgccct cccatcagtg   | 1800 |
| gacaaattag atgttcacat aatattacag ggctgctatt aacaagagat ggtggtaata  | 1860 |
| gcaacaatga gtccgagatc ttcagacctg gaggaggaga tatgaggagac aattggagaa | 1920 |
| gtgaattata taaatataaa gtagtaaaaa ttgaaccatt aggagtagca cccaccaagg  | 1980 |
| caaagagaag agtgggtgag agagaaaaa gagcagtggg aataggagct ttgttccttg   | 2040 |
| ggttcttggg agcagcagga agcactatgg gcgcagcgtc aatgacgctg acggtacagg  | 2100 |
| ccagacaatt attgtctggt atagtgcagc agcagaacaa tttgctgagg gctattgagg  | 2160 |
| cgcaacagca tctgttgcaa ctcacagtct ggggcatcaa gcagctccag gcaagaatcc  | 2220 |
| tggtcttgga aagataccta aaggatcaac agctcctggg gatttggggg tgctctggaa  | 2280 |
| aactcatttg caccactgct gtgccttgga atgctagtgt gagtaataaa tctctggaac  | 2340 |
| agatttgga taacatgacc tggatggagt gggacagaga aattaacaat tacacaagct   | 2400 |
| taatacactc cttaattgaa gaatcgcaaa accagcaaga aaagaatgaa caagaattat  | 2460 |
| tggaattaga taaatgggca agtttgtgga attggtttaa cataacaaat tggctgtggt  | 2520 |
| atataaaatt attcataatg atagtaggag gcttggtagg tttagaata gtttttctg    | 2580 |
| tactttctgt agtgaataga gttaggcagg gatattcacc attatcggtt cagaccacc   | 2640 |
| tcccaatccc gaggggaccc gacaggcccc aaggaataga agaagaagg ggagagagag   | 2700 |
| acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg gacgatctgc  | 2760 |
| ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt gtaacgagga  | 2820 |
| ttgtggaact tctgggacgc agggggtggg aagccctcaa atattgggtg aatctcctac  | 2880 |
| agtattggag tcaggagcta aagaatagtg ctgttagctt gctcaatgcc acagctatag  | 2940 |
| cagtagctga ggggacagat aggggttatag aagtagtaca aggagcttat agagctattc | 3000 |
| gccacatacc tagaagaata agacagggct tggaaaggat tttgctataa gatgggtggc  | 3060 |
| aagtggtaaa aaagtagtgt ggttggtagg cctgctgtaa gggaaagaat gagacgagct  | 3120 |

## Genphar.740.ST25.txt

gagccagcag cagatggggt gggagcagca tctcgag

3157

&lt;210&gt; 15

&lt;211&gt; 508

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified IL-2

&lt;400&gt; 15

|   |     |
|---|-----|
| tcactctctt taatcactac tcacagtaac ctcaactcct gccacaatgt acaggatgca | 60  |
| actcctgtct tgcattgcac taagtcttgc acttgtcaca aacagtgcac ctacttcaag | 120 |
| ttctacaaag aaaacacagc tacaactgga gcatttactg ctggatttac agatgatttt | 180 |
| gaatggaatt aataattaca agaatcccaa actcaccagg atgctcacat ttaagtttta | 240 |
| catgccaag aaggccacag aactgaaaca tcttcagtgt cttgaagaag aactcaaacc  | 300 |
| tctggaggaa gtgctaaatt tagctcaaag caaaaacttt cacttaagac ccagggactt | 360 |
| aatcagcaat atcaacgtaa tagttctgga actaaaggga tctgaaacaa cattcatgtg | 420 |
| tgaatatgct gatgagacag caaccattgt agaatttctg aacagatgga ttaccttttg | 480 |
| tcaaagcatc atctcaacac taacttga                                    | 508 |

&lt;210&gt; 16

&lt;211&gt; 2280

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env

&lt;400&gt; 16

|  |     |
|--|-----|
| gaattcgcca ccatgggagt gaaggagaaa tatcagcact tgtggagatg ggggtggaga  | 60  |
| tggggcacca tgctccttgg gatgttgatg atctgtagtg ctacagaaaa attgtgggtc  | 120 |
| acagtctatt atgggggtacc tgtgtggaag gaagcaacca ccactctatt ttgtgcatca | 180 |
| gatgctaaag catatgatac agaggtacat aatgtttggg ccacacatgc ctgtgtaccc  | 240 |
| acagacccca acccacaaga agtagtattg gtaaattgtga cagaaaattt taacatgtgg | 300 |



Genphar.740.ST25.txt

|            |             |             |             |            |             |      |
|------------|-------------|-------------|-------------|------------|-------------|------|
| aaaaatgaca | tggtagaaca  | gatgcatgag  | gatataatca  | gtttatggga | tcaaagccta  | 360  |
| aagccatgtg | taaaattaac  | cccactctgt  | gttagtttaa  | agtgcactga | tttgaagaat  | 420  |
| gatactaata | ccaatagtag  | tagcgggaga  | atgataatgg  | agaaaggaga | gataaaaaac  | 480  |
| tgctctttca | atatcagcac  | aagcataaga  | ggtaagggtgc | agaaagaata | tgcatTTTTT  | 540  |
| tataaacttg | atataatacc  | aatagataat  | gatactacca  | gctatacggt | gacaagttgt  | 600  |
| aacacctcag | tcattacaca  | ggcctgtcca  | aagggtatcct | ttgagccaat | tcccatacat  | 660  |
| tatttgcccc | cggctggitt  | tcggtattcta | aaatgtaata  | ataagacggt | caatggaaca  | 720  |
| ggaccatgta | caaagtgcag  | cacagtacaa  | tgtacacatg  | gaattaggcc | agtagtatca  | 780  |
| actcaactgc | tgttaaatgg  | cagtctggca  | gaagaagagg  | tagtaattag | atctgccaat  | 840  |
| ttcacagaca | atgctaaaac  | cataatagta  | cagctgaacc  | aatctgtaga | aattaattgt  | 900  |
| acaagaccca | acaacaatac  | aagaaaaagt  | atccgtatcc  | agagaggacc | agggagagca  | 960  |
| tttgttacaa | taggaaaaat  | aggaaatatg  | agacaagcac  | attgtaacat | tagtagagca  | 1020 |
| aaatggaata | acactttaaa  | acagatagat  | agcaaattaa  | gagaacaatt | tggaaataat  | 1080 |
| aaaacaataa | tctttaagca  | gtcctcagga  | ggggaccag   | aaattgtaac | gcacagtttt  | 1140 |
| aattgtggag | gggaattttt  | ctactgtaat  | tcaacacaac  | tgtttaatat | tacttggttt  | 1200 |
| aatagtactt | ggagtactaa  | agggtcaaat  | aacactgaag  | gaagtgcac  | aatcaccttc  | 1260 |
| ccatgcagaa | taaaacaaat  | tataaacatg  | tggcaggaag  | taggaaaagc | aatgtatgcc  | 1320 |
| cctcccatca | gtggacaaat  | tagatgttca  | tcaaataatta | cagggctgct | attaacaaga  | 1380 |
| gatggtggt  | atagcaacaa  | tgagtccgag  | atcttcagac  | ctggaggagg | agatatgagg  | 1440 |
| gacaattgga | gaagtgaatt  | atataaatat  | aaagtagtaa  | aaattgaacc | attaggagta  | 1500 |
| gcaccaccca | aggcaaagag  | aagagtgggt  | cagactagt   | cagtgggaat | aggagctttg  | 1560 |
| ttccttgggt | tcttgggagc  | agcaggaagc  | actatgggag  | cagcgtcaat | gacgctgacg  | 1620 |
| gtacaggcca | gacaattatt  | gtctggtata  | gtgcagcagc  | agaacaattt | gctgagggct  | 1680 |
| attgaggcgc | aacagcatct  | gttgcaactc  | acagtctggg  | gcatcaagca | gctccaggca  | 1740 |
| agaatcctgg | ctgtggaaag  | atacctaaag  | gatcaacagc  | tcctggggat | ttggggttgc  | 1800 |
| tctggaaaac | tcatttgcac  | cactgctgtg  | ccttgggaatg | ctagtgggag | taataaatct  | 1860 |
| ctggaacaga | tttggaaata  | catgacctgg  | atggagtggg  | acagagaaat | taacaattac  | 1920 |
| acaagcttaa | tacactcctt  | aattgaagaa  | tcgcaaaacc  | agcaagaaaa | gaatgaacaa  | 1980 |
| gaattattgg | aattagataa  | atgggcaagt  | ttgtggaatt  | ggtttaacat | aacaaattgg  | 2040 |
| ctgtggtata | taaaattatt  | cataatgata  | gtaggaggct  | tggtagggtt | aagaatagtt  | 2100 |
| tttgctgtac | tttctgtagt  | gaatagagtt  | aggcagggat  | attcaccatt | atcgtttcag  | 2160 |
| accacctcc  | caatccccgag | gggacccgac  | aggcccgaag  | gaatagaaga | agaagggtgga | 2220 |

## Genphar.740.ST25.txt

gagagagaca gagacagatc cattcgatta gtgaacggat ccttagcact tatctggtaa 2280

<210> 17

<211> 1496

<212> DNA

<213> Artificial sequence

<220>

<223> Full length Gag

<400> 17

ggctagaagg agagaggatg ggtgcgagag cgctcagtatt aagcggggga gaattagatc 60  
 gatgggaaaa aattcggtta aggccagggg gaaagaaaa atataaatta aaacatatag 120  
 tatgggcaag cagggagcta gaacgactac aaccatccct tcagacagga tcagaagaac 180  
 ttagatcatt atataatata gtagcaaccc tctattgtgt gcatcaaagg atagagataa 240  
 aagacaccaa ggaagcttta gacaagatag aggaagagca aaacaaaagt aagaaaaaag 300  
 cacagcaagc agcagctgac acaggacaca gcagtcaggt cagccaaaat taccctatag 360  
 tgcagaacat ccaggggcaa atggtacatc aggccatc acctagaact ttaaatgcat 420  
 gggtaaaagt agtagaagag aaggctttca gcccagaagt aatacccatg ttttcagcat 480  
 tatcagaagg agccacccca caagatttaa acaccatgct aaacacagtg gggggacatc 540  
 aagcagccat gcaaatgtta aaagagacca tcaatgagga agctgcagaa tgggatagag 600  
 tacatccagt gcatgcaggg cctattgcac caggccagat gagagaacca aggggaagtg 660  
 acatagcagg aactactagt acccttcagg aacaaatagg atggatgaca aataatccac 720  
 ctatcccagt aggagaaatt tataaaagat ggataatcct gggattaaat aaaatagtaa 780  
 gaatgtatag ccctaccagc attctggaca taagacaagg accaaaagaa ccttttagag 840  
 actatgtaga ccggttctat aaaactctaa gagccgagca agcttcacag gaggtaaaaa 900  
 attggatgac agaaaccttg ttggtccaaa atgcgaaccc agattgtaag actattttta 960  
 aagcattggg accagcggct acactagaag aaatgatgac agcatgtcag ggagtaggag 1020  
 gacccggcca taaggcaaga gttttggctg aagcaatgag ccaagtaaca aatacagcta 1080  
 ccataatgat gcagagaggc aatttttagga accaaagaaa gatgggtaag tgtttcaatt 1140  
 gtggcaaga agggcacaca gccagaaatt gcagggcccc taggaaaaag ggctgttgga 1200  
 aatgtggaag ggaaggacac caaatgaaag attgtactga gagacaggct aatttttttag 1260  
 ggaagatctg gccttcctac aagggaaggc cagggaattt tcttcagagc agaccagagc 1320

Genphar.740.ST25.txt  
 caacagcccc accatttctt cagagcagac cagagccaac agccccacca gaagagagct 1380  
 tcaggtctgg ggtagagaca acaactcccc ctgagaagca ggagccgata gacaaggaac 1440  
 tgtatccttt aacttcctc agatcactct ttggcaacga cccctcgtca caataa 1496

<210> 18

<211> 492

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 18

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp  
 1 5 10 15  
 Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys  
 20 25 30  
 His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Leu Gln Pro Ser Leu  
 35 40 45  
 Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr  
 50 55 60  
 Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp Thr Lys Glu Ala  
 65 70 75 80  
 Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln  
 85 90 95  
 Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val Ser Gln Asn Tyr  
 100 105 110  
 Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile Ser  
 115 120 125  
 Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe  
 130 135 140  
 Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
 145 150 155 160  
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala  
 165 170 175

Genphar.740.ST25.txt  
 Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp  
                   180                  185                  190

Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met  
           195                  200                  205

Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln  
       210                  215                  220

Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu  
       225                  230                  235                  240

Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met  
                   245                  250                  255

Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro  
                   260                  265                  270

Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln  
           275                  280                  285

Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
       290                  295                  300

Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala  
       305                  310                  315                  320

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro  
                   325                  330                  335

Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn  
                   340                  345                  350

Thr Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys  
           355                  360                  365

Met Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Thr Ala Arg Asn  
       370                  375                  380

Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly  
       385                  390                  395                  400

His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys  
                   405                  410                  415

Ile Trp Pro Ser Tyr Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg  
           420                  425                  430

## Genphar.740.ST25.txt

Pro Glu Pro Thr Ala Pro Pro Phe Leu Gln Ser Arg Pro Glu Pro Thr  
 435 440 445

Ala Pro Pro Glu Glu Ser Phe Arg Ser Gly Val Glu Thr Thr Thr Pro  
 450 455 460

Pro Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Thr Ser  
 465 470 475 480

Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln  
 485 490

<210> 19

<211> 2941

<212> DNA

<213> Artificial sequence

<220>

<223> Modified Env from HIV strain pNL4-3

<400> 19

|  |     |
|--|-----|
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| gcgttactcg acagaggaga gcaagaaatg gagccagtag atcctagact agagccctgg  | 120 |
| aagcatccag gaagtcagcc taaaactgct tgtaccaatt gctattgtaa aaagtgttgc  | 180 |
| tttcattgcc aagtttgttt catgacaaaa gccttaggca tctcctatgg caggaagaag  | 240 |
| cggagacagc gacgaagagc tcatcagaac agtcagactc atcaagcttc tctatcaaag  | 300 |
| cagtaagtag tacatgtaat gcaacctata atagtagcaa tagtagcatt agtagtagca  | 360 |
| ataataatag caatagtgtg gtggtccata gtaatcatag aatataggaa aatattaaga  | 420 |
| caaagaaaaa tagacagggtt aattgataga ctaatagaaa gagcagaaga cagtggcaat | 480 |
| gagagtgaag gagaagtatc agcacttgctg gagatggggg tggaaatggg gcaccatgct | 540 |
| ccttgggata ttgatgatct gtagtgctac agaaaaattg tgggtcacag tctattatgg  | 600 |
| ggtacctgtg tggaaggaag caaccaccac tctattttgt gcatcagatg ctaaagcata  | 660 |
| tgatacagag gtacataatg ttggggccac acatgcctgt gtaccacag accccaaccc   | 720 |
| acaagaagta gtattggtaa atgtgacaga aaattttaac atgtggaaaa atgacatggt  | 780 |
| agaacagatg catgaggata taatcagttt atgggatcaa agcctaaagc catgtgtaaa  | 840 |
| attaacccca ctctgtgtta gtttaaagtg cactgatttg aagaatgata ctaataccaa  | 900 |
| tagtagtagc gggagaatga taatggagaa aggagagata aaaaactgct ctttcaatat  | 960 |

## Genphar.740.ST25.txt

```

cagcacaagc ataagagata aggtgcagaa agaatatgca ttcttttata aacttgatat 1020
agtaccaata gataatacca gctatagggt gataagttgt aacacctcag tcattacaca 1080
ggcctgtcca aaggatccct ttgagccaat tcccatatcat tattgtgccc cggctggttt 1140
tgcgattcta aaatgtaata ataagacgtt caatggaaca ggacctgta caaatgtcag 1200
cacagtacaa tgtacacatg gaatcaggcc agtagtatca actcaactgc tgttaaatgg 1260
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cataatagta cagctgaaca catctgtaga aattaattgt acaagacca acaacaatac 1380
aagaaaaagt atccgtatcc agaggggacc agggagagca ttgtttaca taggaaaaat 1440
aggaaatatg agacaagcac attgtaacat tagtagagca aaatggaatg ccactttaa 1500
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ctactgtaat tcaacacaac tgtttaatag tacttggttt aatagtactt ggagtactga 1680
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tataaacatg tggcaggaag taggaaaagc aatgtatgcc cctcccatca gtggacaaat 1800
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tgggtccgag atcttcagac ctggaggagg cgatatgagg gacaattgga gaagtgaatt 1920
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cactgctgtg ccttggaatg ctagtggag taataaatct ctggaacaga tttggaataa 2340
catgacctgg atggagtggg acagagaaat taacaattac acaagcttaa tacactcctt 2400
aattgaagaa tcgcaaaacc agcaagaaaa gaatgaacaa gaattattgg aattagataa 2460
atgggcaagt ttgtggaatt ggtttaacat acaaattgg ctgtggtata taaaattatt 2520
cataatgata gtaggaggct tggtaggttt aagaatagtt ttgctgtac tttctatagt 2580
gaatagagtt aggcagggat attcaccatt atcgtttcag acccacctcc caatcccag 2640
gggacccgac aggccgaag gaatagaaga agaaggtgga gagagagaca gagacagatc 2700
cattcgatta gtgaacggat ccttagcact tatctgggac gatctgcgga gcctgtgcct 2760
cttcagctac caccgcttga gagacttact cttgattgta acgaggattg tggaaacttct 2820

```

Genphar.740.ST25.txt

|  |      |
|--|------|
| gggacgcagg ggggtgggaag ccctcaaata ttggtggaat ctccctacagt attggagtc | 2880 |
| ggaactaaag aatagtgtctg ttaacttgct caatgccaca gccatagcag tagctgagta | 2940 |
| a  | 2941 |

<210> 20

<211> 2746

<212> DNA

<213> Artificial sequence

<220>

<223> Modified Env/Tat/Rev from pNL4-3

<400> 20

|  |      |
|--|------|
| gaattctgca acaactgctg tttatccatt tcagaattgg gtgtcgacat agcagaatag  | 60   |
| gcgttactcg acagaggaga gcaagaaatg gagccagtag atcctagact agagccctgg  | 120  |
| aagcatccag gaagtcagcc taaaactgct tgtaccaatt gctattgtaa aaagtgttgc  | 180  |
| tttcattgcc aagtttgttt catgacaaaa gccttaggca tctcctatgg caggaagaag  | 240  |
| cggagacagc gacgaagagc tcacagaac agtcagactc atcaagcttc tctatcaaag   | 300  |
| cagtaagtag tacatgtaat gcaacctata atagtagcaa tagtagcatt agtagtagca  | 360  |
| ataataatag caatagttgt gtggtccata gtaatcatag aatataggaa aatattaaga  | 420  |
| caaagaaaaa tagacagggt aattgataga ctaatagaaa gagcagaaga cagtggcaat  | 480  |
| gagagtgaag gagaagtatc agcacttggt gagatggggg tggaaatggg gcaccatgct  | 540  |
| ccttgggata ttgatgatct gtagtgctac agaaaaattg tgggtcacag tctattatgg  | 600  |
| ggtacctgtg tggaagggaag caaccaccac tctattttgt gcatcagatg ctaaagcata | 660  |
| tgatacagag gtacataatg tttgggccac acatgcctgt gtaccacag accccaaccc   | 720  |
| acaagaagta gtattggtaa atgtgacaga aaattttaac atgtggaaaa atgacatggt  | 780  |
| agaacagatg catgaggata taatcagttt atgggatcaa agcctaaagc catgtgtaaa  | 840  |
| attaaccca ctctgtgtta gttgtaacac ctcaagtcatt acacaggcct gtccaaagggt | 900  |
| atcctttgag ccaattccca tacattattg tgccccggct ggttttgcga ttctaaaatg  | 960  |
| taataataag acgttcaatg gaacaggacc atgtacaaat gtcagcacag tacaatgtac  | 1020 |
| acatggaatc aggccagtag tatcaactca actgctgtta aatggcagtc tagcagaaga  | 1080 |
| agatgtagta attagatctg ccaatttcac agacaatgct aaaaccataa tagtacagct  | 1140 |
| gaacacatct gtagaaatta attgtacaag acccaacaac aatacaagaa aaagtatccg  | 1200 |
| tatccagagg ggaccaggga gagcatttgt tacaatagga aaaataggaa atatgagaca  | 1260 |

## Genphar.740.ST25.txt

```

agcacattgt aacattagta gagcaaatg gaatgccact ttaaacaga tagctagcaa 1320
attaagagaa caatttggaa ataataaaac aataatcttt aagcaatcct caggagggga 1380
cccagaaatt gtaacgcaca gttttaattg tggaggggaa tttttctact gtaattcaac 1440
acaactgttt aatagtactt ggtttaatag tacttggagt actgaagggt caaataaacac 1500
tgaaggaagt gacacaatca cactcccatg cagaataaaa caatttataa acatgtggca 1560
ggaagtagga aaagcaatgt atgcccctcc catcagtggg caaattagat gttcatcaaa 1620
tattactggg ctgctattaa caagagatgg tggtaataac aacaatgggt ccgagatctt 1680
cagacctgga ggaggcgata tgagggacaa ttggagaagt gaattatata aatataaagt 1740
agtaaaaatt gaaccattag gagtagcacc caccaaggca aagagaagag tggcgcagac 1800
tagtgcagtg ggaataggag ctttgttcct tgggttcttg ggagcagcag gaagcactat 1860
gggctgcacg tcaatgacgc tgacggtaca ggccagacaa ttattgtctg atatagtgca 1920
gcagcagaac aatttgtgta gggctattga ggcgaacag catctgttgc aactcacagt 1980
ctggggcatc aaacagctcc aggcaagaat cctggctgtg gaaagatacc taaaggatca 2040
acagctcctg gggatttggg gttgctctgg aaaactcatt tgcaccactg ctgtgccttg 2100
gaatgctagt tggagtaata aatctctgga acagatttgg aataacatga cctggatgga 2160
gtgggacaga gaaattaaca attacacaag cttaatacac tccttaattg aagaatcgca 2220
aaaccagcaa gaaaagaatg aacaagaatt attggaatta gataaatggg caagtttgtg 2280
gaattggttt aacataacaa attggctgtg gtatataaaa ttattcataa tgatagtagg 2340
aggcttggtg ggtttaagaa tagtttttgc tgtactttct atagtgaata gagttaggca 2400
gggatattca ccattatcgt ttcagaccca cctcccaatc ccgaggggac ccgacaggcc 2460
cgaaggaata gaagaagaag gtggagagag agacagagac agatccattc gattagtga 2520
cggatcctta gcacttatct gggacgatct gcggagcctg tgcctcttca gctaccaccg 2580
cttgagagac ttactcttga ttgtaacgag gattgtggaa cttctgggac gcaggggggtg 2640
ggaagccctc aaatattggt ggaatctcct acagtattgg agtcaggaac taaagaatag 2700
tgctgttaac ttgctcaatg ccacagccat agcagtagct gagtaa 2746

```

&lt;210&gt; 21

&lt;211&gt; 3417

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;



## Genphar.740.ST25.txt

&lt;223&gt; Modified Env/Tat/Rev/Nef from strain BH10

&lt;400&gt; 21

```

gaattctgca acaactgctg tttatccatt ttcagaattg ggtgtcgaca tagcagaata      60
ggcgttactc gacagaggag agcaagaaat ggagccagta gatcctagac tagagccctg     120
gaagcatcca ggaagtcagc ctaaaactgc ttgtaccaat tgctattgta aaaagtgttg     180
ctttcattgc caagtttggt tcataacaaa agccttaggc atctcctatg gcaggaagaa     240
gcggagacag cgacgaagac ctctcaagg cagtcagact catcaagttt ctctatcaaa     300
gcagtaagta gtacatgtaa tgcaacctat acaaatagca atagtagcat tagtagtagc     360
aataataata gcaatagttg tgtggtccat agtaatcata gaatatagga aaatattaag     420
acaaagaaaa atagacaggt taattgatag actaatagaa agagcagaag acagtggcaa     480
tgagagtgaa ggagaaatat cagcacttgt ggagatgggg gtggagatgg ggcaccatgc     540
tccttgggat gttgatgatc tgtagtgcta cagaaaaatt gtgggtcaca gtctattatg     600
gggtacctgt gtggaaggaa gcaaccacca ctctattttg tgcatacagat gctaaagcat     660
atgatacaga ggtacataat gtttgggcca cacatgcctg tgtaccaca gacccaacc      720
cacaagaagt agtattggta aatgtgacag aaaattttta catgtggaaa aatgacatgg     780
tagaacagat gcatgaggat ataatcagtt tatgggatca aagcctaaag ccatgtgtaa     840
aattaacccc actctgtggt agtttaaagt gcactgattt gaagaatgat actaatacca     900
atagtagtag cgggagaatg ataatggaga aaggagagat aaaaaactgc tctttcaata     960
tcagcacaag cataagaggt aagggtgcaga aagaatatgc atttttttat aaacttgata    1020
taataccaat agataatgat actaccagct atacgttgac aagttgtaac acctcagtca    1080
ttacacaggc ctgtccaaag gtatcctttg agccaattcc catacattat tgtgccccgg    1140
ctggttttgc gattctaaaa tgtaataata agacgttcaa tggaacagga ccatgtacaa    1200
atgtcagcac agtacaatgt acacatggaa ttaggccagt agtatcaact caactgctgt    1260
taaattggcag tctggcagaa gaagaggtag taattagatc tgccaatttc acagacaatg    1320
ctaaaaccat aatagtacag ctgaaccaat ctgtagaaat taattgtaca agaccaaca    1380
acaatacaag aaaaagtatc cgtatccaga gaggaccagg gagagcattt gttacaatag    1440
gaaaaatagg aaatatgaga caagcacatt gtaacattag tagagcaaaa tggaataaca    1500
ctttaaaaca gatagatagc aaattaagag aacaatttgg aaataataaa acaataatct    1560
ttaagcagtc ctcaggaggg gaccagaaa ttgtaacgca cagttttaat tgtggagggg    1620
aatttttcta ctgtaattca acacaactgt ttaatagtag ttggtttaat agtacttggg    1680
gtactaaagg gtcaaataac actgaaggaa gtgacacaat caccctccca tgcagaataa    1740
aacaatttat aaacatgtgg caggaagtag gaaaagcaat gtatgcccct cccatcagtg    1800

```

## Genphar.740.ST25.txt

gacaaattag atgttcatca aatattacag ggctgctatt aacaagagat ggtggtaata 1860  
 gcaacaatga gtccgagatc ttcagacctg gaggaggaga tatgaggagac aattggagaa 1920  
 gtgaattata taaatataaa gtagtaaaaa ttgaaccatt aggagtagca cccaccaagg 1980  
 caaagagaag agtgggtgcag actagtgcag tgggaatagg agctttgttc cttgggttct 2040  
 tgggagcagc aggaagcact atgggcgagc cgtcaatgac gctgacggtg caggccagac 2100  
 aattattgtc tggatatagt cagcagcaga acaatttgct gagggctatt gaggcgcaac 2160  
 agcatctgtt gcaactcaca gtctggggca tcaagcagct ccaggcaaga atcctggctg 2220  
 tggaaagata cctaaaggat caacagctcc tggggatttg gggttgctct ggaaaactca 2280  
 tttgcaccac tgctgtgcct tggaaatgcta gttggagtaa taaatctctg gaacagattt 2340  
 ggaataacat gacctggatg gagtgggaca gagaaattaa caattacaca agcttaatac 2400  
 actccttaat tgaagaatcg caaaaccagc aagaaaagaa tgaacaagaa ttattggaat 2460  
 tagataaatg ggcaagtttg tggaaattggt ttaacataac aaattggctg tggtatataa 2520  
 aattattcat aatgatagta ggaggcttgg taggtttaag aatagttttt gctgtacttt 2580  
 ctgtagtgaa tagagttagg cagggatatt caccattatc gtttcagacc cacctcccaa 2640  
 tcccgagggg acccgacagg cccgaaggaa tagaagaaga aggtggagag agagacagag 2700  
 acagatccat tcgattagtg aacggatcct tagcacttat ctgggacgat ctgcggagcc 2760  
 tgtgcctctt cagctaccac cgcttgagag acttactctt gattgtaacg aggattgtgg 2820  
 aacttctggg acgcaggggg tgggaagccc tcaaatattg gtggaatctc ctacagtatt 2880  
 ggagtcagga gctaaagaat agtgctgtta gcttgctcaa tgccacagct atagcagtag 2940  
 ctgaggggac agatagggtt atagaagtag tacaaggagc ttatagagct attcgccaca 3000  
 tacctagaag aataagacag ggcttggaag ggattttgct ataagatggg tggcaagtgg 3060  
 tcaaaaagta gtgtggttgg atggcctgct gtaagggaaa gaatgagacg agctgagcca 3120  
 gcagcagatg ggggtgggagc agcatctcga gacctagaaa aacatggagc aatcacaagt 3180  
 agcaacacag cagctaaca tgctgattgt gcctggctag aagcacaaga ggaggaggag 3240  
 gtgggttttc cagtcacacc tcaggtacct ttaagaccaa tgacttaca ggcagctgta 3300  
 gatcttagcc actttttaa agaaaagggg ggactggaag ggctaattca ctcccaacga 3360  
 agacaagata tccttgatct gtggatctac cacacacaag gctacttccc tgattag 3417

&lt;210&gt; 22

&lt;211&gt; 2950

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

## Genphar.740.ST25.txt

&lt;220&gt;

&lt;223&gt; Modified Env/Nef from strain BH10

&lt;400&gt; 22

|  |      |
|--|------|
| gaattcgcca ccatgggagt gaaggagaaa tatcagcact tgtggagatg ggggtggaga  | 60   |
| tggggcacca tgctccttgg gatgttgatg atctgtagtg ctacagaaaa attgtgggtc  | 120  |
| acagtctatt atgggggtacc tgtgtggaag gaagcaacca ccactctatt ttgtgcatca | 180  |
| gatgctaaag catatgatac agaggtacat aatgtttggg ccacacatgc ctgtgtaccc  | 240  |
| acagacccca acccacaaga agtagtattg gtaaattgtga cagaaaattt taacatgtgg | 300  |
| aaaaatgaca tggtagaaca gatgcatgag gatataatca gtttatggga tcaaagccta  | 360  |
| aagccatgtg taaaattaac cccactctgt gttagtttaa agtgactga tttgaagaat   | 420  |
| gatactaata ccaatagtag tagcgggaga atgataatgg agaaaggaga gataaaaaac  | 480  |
| tgctctttca atatcagcac aagcataaga ggtaagggtgc agaaagaata tgcatttttt | 540  |
| tataaacttg atataatacc aatagataat gatactacca gctatacgtt gacaagttgt  | 600  |
| aacacctcag tcattacaca ggcctgtcca aagggtatcct ttgagccaat tcccatacat | 660  |
| tattgtgccc cggctggttt tgcgattcta aaatgtaata ataagacgtt caatggaaca  | 720  |
| ggaccatgta caaatgtcag cacagtacaa tgtacacatg gaattaggcc agtagtatca  | 780  |
| actcaactgc tgttaaatgg cagtctggca gaagaagagg tagtaattag atctgccaat  | 840  |
| ttcacagaca atgctaaaac cataatagta cagctgaacc aatctgtaga aattaattgt  | 900  |
| acaagaccca acaacaatac aagaaaaagt atccgtatcc agagaggacc agggagagca  | 960  |
| tttgttacaa taggaaaaat aggaaatatg agacaagcac attgtaacat tagtagagca  | 1020 |
| aaatggaata acactttaaa acagatagat agcaaattaa gagaacaatt tggaaataat  | 1080 |
| aaaacaataa tctttaagca gtcctcagga ggggaccag aaattgtaac gcacagtgtt   | 1140 |
| aattgtggag ggggaatttt ctactgtaat tcaacacaac tgtttaatag tacttggttt  | 1200 |
| aatagtactt ggagtactaa aggggtcaaat aacactgaag gaagtgcac aatcacctc   | 1260 |
| ccatgcagaa taaaacaaat tataaacatg tggcaggaag taggaaaagc aatgtatgcc  | 1320 |
| cctcccatca gtggacaaat tagatgttca tcaaatatta cagggtgct attaacaaga   | 1380 |
| gatggtggtg atagcaacaa tgagtccgag atcttcagac ctggaggagg agatatgagg  | 1440 |
| gacaattgga gaagtgaatt atataaatat aaagtagtaa aaattgaacc attaggagta  | 1500 |
| gcacccacca aggcaaagag aagagtgggtg cagactagtg cagtgggaat aggagctttg | 1560 |
| ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat gacgctgacg  | 1620 |
| gtacaggcca gacaattatt gtctgggtata gtgcagcagc agaacaattt gctgagggt  | 1680 |

## Genphar.740.ST25.txt

```

attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca gctccaggca 1740
agaatcctgg ctgtggaag atacctaaag gatcaacagc tcctggggat ttggggttgc 1800
tctgaaaaac tcatttgac cactgctgtg ccttggaatg ctagttggag taataaatct 1860
ctggaacaga tttggaataa catgacctgg atggagtggg acagagaaat taacaattac 1920
acaagcttaa tacactcctt aattgaagaa tcgcaaaacc agcaagaaaa gaatgaacaa 1980
gaattattgg aattagataa atgggcaagt ttgtggaatt ggtttaacat aacaaattgg 2040
ctgtggtata taaaattatt cataatgata gtaggaggct tggtaggttt aagaatagtt 2100
tttgctgtac tttctgtagt gaatagagtt aggcagggat attcaccatt atcgtttcag 2160
accacacctc caatcccag gggacccgac agggccgaag gaatagaaga agaagggtga 2220
gagagagaca gagacagatc cattcgatta gtgaacggat ccttagcact tatctgggac 2280
gatctgcgga gcctgtgcct cttcagctac caccgcttga gagacttact cttgattgta 2340
acgaggattg tggaacttct gggacgcagg ggggtgggaag ccctcaaata ttggtggaat 2400
ctcctacagt attggagtca ggagctaaag aatagtgtg ttagcttgct caatgccaca 2460
gctatagcag tagctgaggg gacagatagg gttatagaag tagtacaagg agcttataga 2520
gctattcgcc acatacctag aagaataaga cagggcttgg aaaggatttt gctataagat 2580
gggtggcaag tgggtcaaaa gtagtgtggt tggatggcct gctgtaaggg aaagaatgag 2640
acgagctgag ccagcagcag atgggggtgg agcagcatct cgagacctag aaaaacatgg 2700
agcaatcaca agtagcaaca cagcagctaa caatgctgat tgtgcctggc tagaagcaca 2760
agaggaggag gaggtgggtt ttccagtcac acctcaggta cttttaagac caatgactta 2820
caaggcagct gtagatctta gccacttttt aaaagaaaag gggggactgg aagggtctaat 2880
tcactcccaa cgaagacaag atatccttga tctgtggatc taccacacac aaggctactt 2940
ccctgattag 2950

```

&lt;210&gt; 23

&lt;211&gt; 2747

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env/Tat from strain BH10

&lt;400&gt; 23

```

gaattctgca acaactgctg tttatccatt ttcagaattg ggtgtcgaca tagcagaata 60

```

Genphar.740.ST25.txt

|   |      |
|---|------|
| ggcgttactc gacagaggag agcaagaaat ggagccagta gatcctagac tagagccctg   | 120  |
| gaagcatcca ggaagtcagc ctaaaactgc ttgtaccaat tgctattgta aaaagtgttg   | 180  |
| ctttcattgc caagtttggt tcataacaaa agccttaggc atctcctatg gcaggaagaa   | 240  |
| gcggagacag cgacgaagac ctcctcaagg cagtcagact catcaagttt ctctatcaaa   | 300  |
| gcagtaagta gtacatgtaa tgcaacctat acaaataagca atagtagcat tagtagtagc  | 360  |
| aataataata gcaatagttg tgtgggtccat agtaatcata gaatatagga aaatattaag  | 420  |
| acaaagaaaa atagacagggt taattgatag actaatagaa agagcagaag acagtggcaa  | 480  |
| tgagagtga ggaagaaat cagcacttgt ggagatgggg gtggagatgg ggcaccatgc     | 540  |
| tccttgggat gttgatgac tgtagtgcta cagaaaaatt gtgggtcaca gtctattatg    | 600  |
| gggtacctgt gtggaaggaa gcaaccacca ctctattttg tgcacagat gctaaagcat    | 660  |
| atgatacaga ggtacataat gtttgggcca cacatgcctg tgtaccaca gacccaacc     | 720  |
| cacaagaagt agtattggta aatgtgacag aaaattttta catgtggaaa aatgacatgg   | 780  |
| tagaacagat gcatgaggat ataatcagtt tatgggatca aagcctaaag ccatgtgtaa   | 840  |
| aattaacccc actctgtgtt agtttaaggt gcaactgatt gaagaatgat actaatacca   | 900  |
| atagtagtag cgggagaatg ataatggaga aaggagagat aaaaaactgc tctttcaata   | 960  |
| tcagcacaag cataagagggt aagggtgcaga aagaatatgc atttttttat aaacttgata | 1020 |
| taataccaat agataatgat actaccagct atacgttgac aagttgtaac acctcagtca   | 1080 |
| ttacacaggc ctgtccaaag gtatcctttg agccaattcc catacattat tgtgccccgg   | 1140 |
| ctggttttgc gattctaaaa tgtaataata agacgttcaa tggaacagga ccatgtacaa   | 1200 |
| atgtcagcac agtacaatgt acacatggaa ttaggccagt agtatcaact caactgctgt   | 1260 |
| taaatggcag tctggcagaa gaagaggtag taattagatc tgccaatttc acagacaatg   | 1320 |
| ctaaaaccat aatagtacag ctgaaccaat ctgtagaaat taattgtaca agaccaaca    | 1380 |
| acaatacaag aaaaagtatc cgtatccaga gaggaccagg gagagcattt gttacaatag   | 1440 |
| gaaaaatagg aaatatgaga caagcacatt gtaacattag tagagcaaaa tggaataaca   | 1500 |
| ctttaaaaca gatagatagc aaattaagag aacaatttgg aaataataaa acaataatct   | 1560 |
| ttaagcagtc ctcaggaggg gaccagaaa ttgtaacgca cagttttaat tgtggagggg    | 1620 |
| aatttttcta ctgtaattca acacaactgt ttaatagtag ttggtttaat agtacttgga   | 1680 |
| gtactaaagg gtcaaataac actgaaggaa gtgacacaat caccctcca tgcagaataa    | 1740 |
| aacaaattat aaacatgtgg caggaagtag gaaaagcaat gtatgccct cccatcagt     | 1800 |
| gacaaattag atgttcacat aatattacag ggctgctatt aacaagagat ggtggaata    | 1860 |
| gcaacaatga gtccgagatc ttcagacctg gaggaggaga tatgaggagc aattggagaa   | 1920 |
| gtgaattata taaatataaa gtagtaaaaa ttgaaccatt aggagtagca cccaccaagg   | 1980 |

## Genphar.740.ST25.txt

caaagagaag agtgggtgcag actagtgcag tgggaatagg agctttgttc cttgggttct 2040  
 tgggagcagc aggaagcact atgggcgagc cgtcaatgac gctgacggtg caggccagac 2100  
 aattattgtc tggatatagt cagcagcaga acaatttgct gagggctatt gaggcgcaac 2160  
 agcatctgtt gcaactcaca gtctggggca tcaagcagct ccaggcaaga atcctggctg 2220  
 tggaaagata cctaaaggat caacagctcc tggggatttg gggttgctct ggaaaactca 2280  
 tttgcaccac tgctgtgcct tggaaatgcta gttggagtaa taaatctctg gaacagattt 2340  
 ggaataacat gacctggatg gagtgggaca gagaaattaa caattacaca agcttaatac 2400  
 actccttaat tgaagaatcg caaaaccagc aagaaaagaa tgaacaagaa ttattggaat 2460  
 tagataaatg ggcaagtttg tggaaattggt ttaacataac aaattggctg tggatatataa 2520  
 aattattcat aatgatagta ggaggcttgg taggtttaag aatagttttt gctgtacttt 2580  
 ctgtagtgaa tagagttagg cagggatatt caccattatc gtttcagacc cacctcccaa 2640  
 tcccgagggg acccgacagg cccgaaggaa tagaagaaga aggtggagag agagacagag 2700  
 acagatccat tcgattagtg aacggatcct tagcacttat ctggttaa 2747

&lt;210&gt; 24

&lt;211&gt; 2583

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env

&lt;400&gt; 24

gaattcgcca ccatgggagt gaaggagaaa tatcagcact tgtggagatg ggggtggaga 60  
 tggggcacca tgctccttgg gatgttgatg atctgtagtg ctacagaaaa attgtgggtc 120  
 acagtctatt atgggttacc tgtgtggaag gaagcaacca ccaactctatt ttgtgcatca 180  
 gatgctaaag catatgatac agaggtagat aatgtttggg ccacacatgc ctgtgtaccc 240  
 acagacccca acccacaaga agtagtattg gtaaatgtga cagaaaattt taacatgtgg 300  
 aaaaatgaca tggtagaaca gatgcatgag gatataatca gtttatggga tcaaagccta 360  
 aagccatgtg taaaattaac ccactctgt gtagtttaa agtgactga tttgaagaat 420  
 gatactaata ccaatagtag tagcgggaga atgataatgg agaaaggaga gataaaaaac 480  
 tgctctttca atatcagcac aagcataaga ggtaagggtgc agaaagaata tgcatttttt 540  
 tataaacttg atataatacc aatagataat gatactacca gctatacggt gacaagttgt 600

Genphar.740.ST25.txt

|  |      |
|--|------|
| aacacctcag tcattacaca ggcctgtcca aaggtatcct ttgagccaat tcccatacat    | 660  |
| tattgtgccc cggctggttt tgcgattcta aaatgtaata ataagacgtt caatggaaca    | 720  |
| ggaccatgta caaatgtcag cacagtacaa tgtacacatg gaattaggcc agtagtatca    | 780  |
| actcaactgc tgttaaatgg cagtctggca gaagaagagg tagtaattag atctgccaat    | 840  |
| ttcacagaca atgctaaaac cataatagta cagctgaacc aatctgtaga aattaattgt    | 900  |
| acaagacca acaacaatac aagaaaaagt atccgtatcc agagaggacc agggagagca     | 960  |
| tttgttacaa taggaaaaat aggaaatatg agacaagcac attgtaacat tagtagagca    | 1020 |
| aaatggaata acactttaaa acagatagat agcaaattaa gagaacaatt tggaaataat    | 1080 |
| aaaacaataa tctttaagca gtcctcagga ggggaccag aaattgtaac gcacagtttt     | 1140 |
| aattgtggag gggaattttt ctactgtaat tcaacacaac tgtttaatag tacttggttt    | 1200 |
| aatagtactt ggagtactaa agggtcaa at aacactgaag gaagtgcac aatcacctc     | 1260 |
| ccatgcagaa taaaacaaat tataaacatg tggcaggaag taggaaaagc aatgtatgcc    | 1320 |
| cctcccatca gtggacaaat tagatgttca tcaaataatta cagggtgctgct attaacaaga | 1380 |
| gatggtggtg atagcaacaa tgagtccgag atcttcagac ctggaggagg agatatgagg    | 1440 |
| gacaattgga gaagtgaatt atataaatat aaagtagtaa aaattgaacc attaggagta    | 1500 |
| gcacccacca aggcaaagag aagagtgggtg cagagagaaa aaagagcagt gggaatagga   | 1560 |
| gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc gtcaatgacg    | 1620 |
| ctgacggtac aggccagaca attattgtct ggtatagtgc agcagcagaa caatttgctg    | 1680 |
| agggctattg aggcgaaca gcatctgttg caactcacag tctggggcat caagcagctc     | 1740 |
| caggcaagaa tcctggctgt ggaaagatac ctaaaggatc aacagctcct ggggatttgg    | 1800 |
| ggttgctctg gaaaactcat ttgcaccact gctgtgcctt ggaatgctag ttggagtaat    | 1860 |
| aaatctctgg aacagatttg gaataacatg acctggatgg agtgggacag agaaattaac    | 1920 |
| aattacacaa gcttaataca ctccttaatt gaagaatcgc aaaaccagca agaaaagaat    | 1980 |
| gaacaagaat tattggaatt agataaatgg gcaagtttgt ggaattggtt taacataaca    | 2040 |
| aattggctgt ggtatataaa attattcata atgatagtag gaggcttgggt aggtttaaga   | 2100 |
| atagtttttg ctgtactttc tgtagtgaat agagttaggc agggatatcc accattatcg    | 2160 |
| tttcagaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat agaagaagaa    | 2220 |
| ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt agcacttatc    | 2280 |
| tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga cttactcttg    | 2340 |
| attgtaacga ggattgtgga acttctggga cgcaggggggt gggaagccct caaatattgg   | 2400 |
| tggaatctcc tacagtattg gagtcaggag ctaaagaata gtgctgttag cttgctcaat    | 2460 |
| gccacagcta tagcagtagc tgaggggaca gatagggtta tagaagtagt acaaggagct    | 2520 |

## Genphar.740.ST25.txt

tatagagcta ttcgccacat acctagaaga ataagacagg gcttggaaag gattttgcta 2580  
taa 2583

<210> 25

<211> 108

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 25  
tgtacaagac ccaacaacaa tacaagaaaa agtatccgta tccagagagg accagggaga 60  
gcatttgcta caataggaaa aataggaaat atgagacaag cacattgt 108

<210> 26

<211> 105

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 26  
tgtaccagac ctaacaacaa tacaagaaaa agtgtacgta taggaccagg acaaacattc 60  
tatgcaacag gtgatataat aggggatata agacaagcac attgt 105

<210> 27

<211> 105

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 27  
tgtacgagac ccaacaataa tacaagaaaa agtataagga taggaccagg acaagcattc 60  
tatgcaacag gagaaataat aggagatata agacaagcac attgt 105

<210> 28

<211> 102

<212> DNA

<213> Human immunodeficiency virus type 1



## Genphar.740.ST25.txt

<400> 28  
tgcacaaggc cctacaacaa tataagacaa aggaccccca taggactagg gcaagcactc 60  
tatacaacaa gaagaataga agatataaga agagcacatt gt 102

<210> 29

<211> 105

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 29  
tgtaccagac cctccaccaa tacaagaaca agtatacgta taggaccagg acaagtattc 60  
tatagaacag gagacataac aggagatata agaaaagcat attgt 105

<210> 30

<211> 105

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 30  
tgtacaagac ccaacaacaa tacaagaaaa agaatatctt taggaccagg acgagtattt 60  
tatacagcag gagaataat aggagacatc agaaaggcac attgt 105

<210> 31

<211> 105

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 31  
tgtaccagac ctaataacaa tacaagaaaa agtataactt ttgcaccagg acaagcgctc 60  
tatgcaacag gtgaaataat aggagatata agacaagcac attgt 105

<210> 32

<211> 2562

<212> DNA

<213> Artificial sequence

Genphar.740.ST25.txt

&lt;220&gt;

&lt;223&gt; Env with multi-clade V3 loops

&lt;400&gt; 32

|  |      |
|--|------|
| atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg  | 60   |
| ctccttgggg tgttgatgat ctgtagtgct acagaaaaat tgtgggtcac agtctattat  | 120  |
| ggggtacctg tgtggaagga agcaaccacc actctatfff gtgcatcaga tgctaaagca  | 180  |
| tatgatacag aggtacataa tgtttgggcc acacatgcct gtgtaccac agacccaac    | 240  |
| ccacaagaag tagtattggt aaatgtgaca gaaaatttta acatgtggaa aaatgacatg  | 300  |
| gtagaacaga tgcagtagga tataatcagt ttatgggatc aaagcctaaa gccatgtgta  | 360  |
| aaattaaccc cactctgtgt tggagctggt agttgtaaca cctcagtcac tacacaggcc  | 420  |
| tgtccaaagg tctccttga gccaatccc atacattatt gtgccccggc tggttttgcg    | 480  |
| attctaaaat gtaataataa gacgttcaat ggaacaggac catgtacaaa tgtcagcaca  | 540  |
| gtacaatgta cacatggaat taggccagta gtatcaactc aactgctgtt aaatggcagt  | 600  |
| ctggcagaag aagaggtagt aattagatct gccaatfca cagacaatgc taaaaccata   | 660  |
| atagtacagc tgaaccaatc tgtagaaatt aattgtacaa gaccaacaa caatacaaga   | 720  |
| aaaagtatcc gtatccagag aggaccaggg agagcatttg ttacaatagg aaaaatagga  | 780  |
| aatatgagac aagcacattg tctcgggtgt accagaccta acaacaatac aagaaaaagt  | 840  |
| gtacgtatag gaccaggaca aacattctat gcaacagggt atataatagg ggatataaga  | 900  |
| caagcacatt gttgtacgag acccaacaat aatacaagaa aaagtataag gataggacca  | 960  |
| ggacaagcat tctatgcaac aggagaaata ataggagata taagacaagc acattgttgc  | 1020 |
| acaaggccct acaacaatat aagacaaagg acccccatag gactagggca agcactctat  | 1080 |
| acaacaagaa gaatagaaga tataagaaga gcacattggt gtaccagacc ctccaccaat  | 1140 |
| acaagaacaa gtatacgtat aggaccagga caagtattct atagaacagg agacataaca  | 1200 |
| ggagatataa gaaaagcata ttgtggatcc tgtacaagac ccaacaacaa tacaagaaaa  | 1260 |
| agaatatctt taggaccagg acgagtattt tatacagcag gagaaataat aggagacatc  | 1320 |
| agaaaggcac attgttgtag cagacctaata aacaatacaa gaaaaagtat aacttttgca | 1380 |
| ccaggacaag cgctctatgc aacaggtgaa ataataggag atataagaca agcacattgt  | 1440 |
| ctcgggaaca ttagtagagc aaaatggaat aacactttaa aacagataga tagcaaatta  | 1500 |
| agagaacaat ttggaaataa taaaacaata atctttaagc agtcctcagg aggggaccca  | 1560 |
| gaaattgtaa cgcacagttt taattgtgga ggggaatttt tctactgtaa ttcaacacaa  | 1620 |
| ctgtttaata gtacttggtt taatagtact tggagtacta aagggtcaaa taacactgaa  | 1680 |

Genphar.740.ST25.txt

```

ggaagtgaca caatcacccct cccatgcaga ataaaacaaa ttataaacat gtggcaggaa 1740
gtaggaaaag caatgtatgc cctccccatc agtggacaaa ttagatgttc atcaaatatt 1800
acagggctgc tattaacaag agatgggtgt aatagcaaca atgagtccga gatcttcaga 1860
cctggaggag gagatatgag ggacaattgg agaagtgaat tatataaata taaagtagta 1920
aaaattgaac cattaggagt agcaccacc aaggcaaaga gaagagtggg gcagactagt 1980
gcagtgggaa taggagcttt gttccttggg ttcttgggag cagcaggaag cactatgggc 2040
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cagaacaatt tgctgagggc tattgaggcg caacagcatc tgttgcaact cacagtctgg 2160
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ctcctgggga tttggggttg ctctggaaaa ctcatttgca cactgctgt gccttggaat 2280
gctagttaga gtaataaatc tctggaacag atttggaata acatgacctg gatggagtgg 2340
gacagagaaa ttaacaatta cacaagctta atacactcct taattgaaga atcgcaaac 2400
cagcaagaaa agaataaaca agaattattg gaattagata aatgggcaag tttgtggaat 2460
tggtttaaca taacaattg gctgtggtat ataaaatcgt ggctgctgct gctcctgctc 2520
tcctctccc tcctccaggc caccgatttc atgtccctgt ga 2562

```

&lt;210&gt; 33

&lt;211&gt; 853

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env with multi-clade V3 loops

&lt;400&gt; 33

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu  
 20 25 30

Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala  
 35 40 45

Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu  
 50 55 60

Genphar.740.ST25.txt

Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn  
65 70 75 80

Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn Phe Asn Met Trp  
85 90 95

Lys Asn Asp Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp  
100 105 110

Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Gly  
115 120 125

Ala Gly Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val  
130 135 140

Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala  
145 150 155 160

Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr  
165 170 175

Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser  
180 185 190

Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val Ile  
195 200 205

Arg Ser Ala Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu  
210 215 220

Asn Gln Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg  
225 230 235 240

Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile  
245 250 255

Gly Lys Ile Gly Asn Met Arg Gln Ala His Cys Leu Gly Cys Thr Arg  
260 265 270

Pro Asn Asn Asn Thr Arg Lys Ser Val Arg Ile Gly Pro Gly Gln Thr  
275 280 285

Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
290 295 300

Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro  
305 310 315 320

## Genphar.740.ST25.txt

Gly Gln Ala Phe Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln  
 325 330 335  
 Ala His Cys Cys Thr Arg Pro Tyr Asn Asn Ile Arg Gln Arg Thr Pro  
 340 345 350  
 Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr Arg Arg Ile Glu Asp Ile  
 355 360 365  
 Arg Arg Ala His Cys Cys Thr Arg Pro Ser Thr Asn Thr Arg Thr Ser  
 370 375 380  
 Ile Arg Ile Gly Pro Gly Gln Val Phe Tyr Arg Thr Gly Asp Ile Thr  
 385 390 395 400  
 Gly Asp Ile Arg Lys Ala Tyr Cys Gly Ser Cys Thr Arg Pro Asn Asn  
 405 410 415  
 Asn Thr Arg Lys Arg Ile Ser Leu Gly Pro Gly Arg Val Phe Tyr Thr  
 420 425 430  
 Ala Gly Glu Ile Ile Gly Asp Ile Arg Lys Ala His Cys Cys Thr Arg  
 435 440 445  
 Pro Asn Asn Asn Thr Arg Lys Ser Ile Thr Phe Ala Pro Gly Gln Ala  
 450 455 460  
 Leu Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
 465 470 475 480  
 Leu Gly Asn Ile Ser Arg Ala Lys Trp Asn Asn Thr Leu Lys Gln Ile  
 485 490 495  
 Asp Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn Lys Thr Ile Ile Phe  
 500 505 510  
 Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val Thr His Ser Phe Asn  
 515 520 525  
 Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr Gln Leu Phe Asn Ser  
 530 535 540  
 Thr Trp Phe Asn Ser Thr Trp Ser Thr Lys Gly Ser Asn Asn Thr Glu  
 545 550 555 560  
 Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn  
 565 570 575

## Genphar.740.ST25.txt

Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Ser Gly  
 580 585 590  
 Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp  
 595 600 605  
 Gly Gly Asn Ser Asn Asn Glu Ser Glu Ile Phe Arg Pro Gly Gly Gly  
 610 615 620  
 Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val  
 625 630 635 640  
 Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys Ala Lys Arg Arg Val  
 645 650 655  
 Val Gln Thr Ser Ala Val Gly Ile Gly Ala Leu Phe Leu Gly Phe Leu  
 660 665 670  
 Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Met Thr Leu Thr Val  
 675 680 685  
 Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu  
 690 695 700  
 Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp  
 705 710 715 720  
 Gly Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala Val Glu Arg Tyr Leu  
 725 730 735  
 Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly Lys Leu Ile  
 740 745 750  
 Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Ser Leu  
 755 760 765  
 Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu Trp Asp Arg Glu Ile  
 770 775 780  
 Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile Glu Glu Ser Gln Asn  
 785 790 795 800  
 Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala  
 805 810 815  
 Ser Leu Trp Asn Trp Phe Asn Ile Thr Asn Trp Leu Trp Tyr Ile Lys

820 Genphar.740.ST25.txt 830  
825 830

Ser Trp Leu Leu Leu Leu Leu Ser Leu Ser Leu Leu Gln Ala Thr  
835 840 845

Asp Phe Met Ser Leu  
850

<210> 34

<211> 1092

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 34  
atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60  
ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag 120  
ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata 180  
ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240  
acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct 300  
ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct 360  
gacacaggac acagcagtca ggtcagccaa aattacccta tagtgcagaa catccagggg 420  
caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa 480  
gagaaggctt tcagcccaga agtaataccc atgttttcag cattatcaga aggagccacc 540  
ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatg 600  
ttaaaagaga ccatcaatga ggaagctgca gaatgggata gagtacatcc agtgcattgca 660  
gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact 720  
agtacccttc aggaacaaat aggatggatg acaataatc cacctatccc agtaggagaa 780  
atttataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc 840  
agcattcttg acataagaca aggacaaaa gaaccttta gagactatgt agaccggttc 900  
tataaaactc taagagccga gcaagcttca caggaggtaa aaaattggat gacagaaacc 960  
ttgttggtcc aaaatgcaaa ccagattgt aagactatgt taaaagcatt gggaccagcg 1020  
gctacactag aagaaatgat gacagcatgt caggagtag gaggaccccg ccataaggca 1080  
agagttttgt aa 1092

<210> 35

Genphar.740.ST25.txt

&lt;211&gt; 1179

&lt;212&gt; DNA

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 35

```

atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg      60
ctccttggga tgttgatgat ctgtagtgtt ggtgctgagag cgtcagtatt aagcggggga      120
gaattagatc gatgggaaaa aattcgggta aggccagggg gaaagaaaaa atataaatta      180
aaacatatag tatgggcaag caggagagcta gaacgattcg cagttaatcc tggcctgtta      240
gaaacatcag aaggctgtag acaaatactg ggacagctac aaccatccct tcagacagga      300
tcagaagaac ttagatcatt atataatata gtagcaaccc tctattgtgt gcatcaaagg      360
atagagataa aagacaccaa ggaagcttta gacaagatag aggaagagca aaacaaaagt      420
aagaaaaaag cacagcaagc agcagctgac acaggacaca gcagtcaggt cagccaaaat      480
taccctatag tgcagaacat ccaggggcaa atggtacatc aggccatata acctagaact      540
ttaaatagcat gggtaaaagt agtagaagag aaggctttca gcccagaagt aatacccatg      600
ttttcagcat tatcagaagg agccacccca caagatttaa acaccatgct aaacacagtg      660
gggggacatc aagcagccat gcaaatgtta aaagagacca tcaatgagga agctgcagaa      720
tgggatagag tacatccagt gcatgcaggg cctattgcac caggccagat gagagaacca      780
aggggaagtg acatagcagg aactactagt acccttcagg aacaaatagg atggatgaca      840
aataatccac ctatcccagt aggagaaatt tataaaagat ggataatcct gggattaaat      900
aaaatagtaa gaatgtatag ccctaccagc attctggaca taagacaagg accaaaagaa      960
ccttttagag actatgtaga ccggttctat aaaactctaa gagccgagca agcttcacag     1020
gaggtaaaaa attggatgac agaaaccttg ttggtccaaa atgcgaaccc agattgtaag     1080
actattttaa aagcattggg accagcggct acactagaag aaatgatgac agcatgtcag     1140
ggagtaggag gacccggcca taaggcaaga gttttgtaa                               1179

```

&lt;210&gt; 36

&lt;211&gt; 1308

&lt;212&gt; DNA

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 36

```

atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg      60

```



Genphar.740.ST25.txt

|  |      |
|--|------|
| ctccttggga tgttgatgat ctgtagtgct ggtgcgagag cgtcagtatt aagcggggga  | 120  |
| gaattagatc gatgggaaaa aattcgggta aggccagggg gaaagaaaaa atataaatta  | 180  |
| aaacatatag tatgggcaag cagggagcta gaacgattcg cagttaatcc tggcctgtta  | 240  |
| gaaacatcag aaggctgtag acaaatactg ggacagctac aaccatccct tcagacagga  | 300  |
| tcagaagaac ttagatcatt atataataga gtagcaaccc tctatttgtg gcatcaaagg  | 360  |
| atagagataa aagacaccaa ggaagcttta gacaagatag aggaagagca aaacaaaagt  | 420  |
| aagaaaaaag cacagcaagc agcagctgac acaggacaca gcagtcaggt cagccaaaat  | 480  |
| taccctatag tgcagaacat ccagggggcaa atggtacatc aggccatatc acctagaact | 540  |
| ttaaatgcat gggtaaaagt agtagaagag aaggctttca gcccagaagt aatacccatg  | 600  |
| ttttcagcat tatcagaagg agccacccca caagatttaa acaccatgct aaacacagtg  | 660  |
| gggggacatc aagcagccat gcaaattgtta aaagagacca tcaatgagga agctgcagaa | 720  |
| tgggatagag tacatccagt gcatgcaggg cctattgcac caggccagat gagagaacca  | 780  |
| aggggaagtg acatagcagg aactactagt acccttcagg aacaaatagg atggatgaca  | 840  |
| aataatccac ctatcccagt aggagaaatt tataaaagat ggataatcct gggattaaat  | 900  |
| aaaatagtaa gaatgtatag ccctaccagc attctggaca taagacaagg accaaaagaa  | 960  |
| ccttttagag actatgtaga ccggttctat aaaactctaa gagccgagca agcttcacag  | 1020 |
| gaggtaaaaa attggatgac agaaaccttg ttggtccaaa atgcgaaccc agattgtaag  | 1080 |
| actattttta aagcattggg accagcggct acactagaag aaatgatgac agcatgtcag  | 1140 |
| ggagtaggag gacccggcca taaggcaaga gttttgttat tcataatgat agtaggaggc  | 1200 |
| ttggtagggt taagaatagt ttttgctgta ctttctgtag tgaatagagt taggcagggg  | 1260 |
| tattcaccat tatcgtttca gaccacctc ccaatcccg ggggataa                 | 1308 |

&lt;210&gt; 37

&lt;211&gt; 363

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 37

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp  
 1 5 10 15

Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys  
 20 25 30

Genphar.740.ST25.txt  
His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro  
35 40 45  
Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu  
50 55 60  
Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn  
65 70 75 80  
Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp  
85 90 95  
Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys  
100 105 110  
Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val  
115 120 125  
Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His  
130 135 140  
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu  
145 150 155 160  
Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser  
165 170 175  
Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly  
180 185 190  
Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu  
195 200 205  
Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala  
210 215 220  
Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr  
225 230 235 240  
Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile  
245 250 255  
Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys  
260 265 270  
Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
275 280 285

Genphar.740.ST25.txt

Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu  
 290 295 300

Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr  
 305 310 315 320

Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala  
 325 330 335

Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly  
 340 345 350

Val Gly Gly Pro Gly His Lys Ala Arg Val Leu  
 355 360

<210> 38

<211> 410

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 38

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Gly Ala  
 20 25 30

Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile  
 35 40 45

Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val  
 50 55 60

Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu  
 65 70 75 80

Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser  
 85 90 95

Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala  
 100 105 110

Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp Thr Lys Glu  
 115 120 125

## Genphar.740.ST25.txt

Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala  
 130 135 140  
 Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val Ser Gln Asn  
 145 150 155 160  
 Tyr Pro Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val Ser  
 165 170 175  
 Gln Asn Tyr Pro Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln  
 180 185 190  
 Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val  
 195 200 205  
 His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val  
 210 215 220  
 Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu  
 225 230 235 240  
 Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val  
 245 250 255  
 Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu  
 260 265 270  
 Glu Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile  
 275 280 285  
 Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr  
 290 295 300  
 Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro  
 305 310 315 320  
 Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn  
 325 330 335  
 Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln  
 340 345 350  
 Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr  
 355 360 365  
 Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Thr Ile Leu Lys Ala Leu

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Genphar.740.ST25.txt

Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val Ser  
165 170 175

Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His Gln  
180 185 190

Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu  
195 200 205

Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu  
210 215 220

Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly  
225 230 235 240

His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala  
245 250 255

Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro  
260 265 270

Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser  
275 280 285

Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro  
290 295 300

Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile  
305 310 315 320

Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro  
325 330 335

Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg  
340 345 350

Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu  
355 360 365

Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu  
370 375 380

Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val  
385 390 395 400

Gly Gly Pro Gly His Lys Ala Arg Val Leu Leu Phe Ile Met Ile Val  
405 410 415

## Genphar.740.ST25.txt

Gly Gly Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu Ser Val Val  
 420 425 430

Asn Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr His Leu  
 435 440 445

Pro Ile Pro Arg Gly  
 450

<210> 40

<211> 399

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 40

|   |     |
|---|-----|
| atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg | 60  |
| ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag | 120 |
| ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata | 180 |
| ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat | 240 |
| acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct | 300 |
| ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct | 360 |
| gacacaggac acagcagtca ggtcagccaa aattactaa                        | 399 |

<210> 41

<211> 486

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 41

|  |     |
|--|-----|
| atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg  | 60  |
| ctccttggga tgttgatgat ctgtagtgct ggtgcgagag cgtcagtatt aagcggggga  | 120 |
| gaattagatc gatgggaaaa aattcgggta aggccagggg gaaagaaaaa atataaatta  | 180 |
| aaacatatag tatgggcaag caggagagcta gaacgattcg cagttaatcc tggcctgtta | 240 |
| gaaacatcag aaggctgtag acaaatactg ggacagctac aaccatccct tcagacagga  | 300 |
| tcagaagaac ttagatcatt atataatata gtagcaaccc tctattgtgt gcatcaaagg  | 360 |
| atagagataa aagacaccaa ggaagcttta gacaagatag _aggaagagca aaacaaaagt | 420 |

## Genphar.740.ST25.txt

aagaaaaaag cacagcaagc agcagctgac acaggacaca gcagtcaggt cagccaaaat 480  
tactaa 486

<210> 42

<211> 615

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 42  
atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg 60  
ctccttggga tgttgatgat ctgtagtgtt ggtgagagag cgtcagtatt aagcggggga 120  
gaattagatc gatgggaaaa aattcgggta aggccagggg gaaagaaaaa atataaatta 180  
aaacatatag tatgggcaag caggagagcta gaacgattcg cagttaatcc tggcctgtta 240  
gaaacatcag aaggctgtag acaaatactg ggacagctac aaccatccct tcagacagga 300  
tcagaagaac ttagatcatt atataatata gtagcaaccc tctatttgtt gcatcaaagg 360  
atagagataa aagacaccaa ggaagcttta gacaagatag aggaagagca aaacaaaagt 420  
aagaaaaaag cacagcaagc agcagctgac acaggacaca gcagtcaggt cagccaaaat 480  
tacttattca taatgatagt aggaggcttg gtaggtttta gaatagtttt tgctgtactt 540  
tctgtagtga atagagttag gcagggatat tcaccattat cgtttcagac ccacctccca 600  
atcccagggg gataa 615

<210> 43

<211> 132

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 43

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp  
1 5 10 15

Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys  
20 25 30

His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro  
35 40 45



## Genphar.740.ST25.txt

Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu  
 50 55 60

Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn  
 65 70 75 80

Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp  
 85 90 95

Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys  
 100 105 110

Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val  
 115 120 125

Ser Gln Asn Tyr  
 130

<210> 44

<211> 179

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 44

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Gly Ala  
 20 25 30

Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile  
 35 40 45

Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val  
 50 55 60

Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu  
 65 70 75 80

Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser  
 85 90 95

Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Gly Gln Leu Gln  
 100 105 110

## Genphar.740.ST25.txt

Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr  
 115 120 125

Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp Thr  
 130 135 140

Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys  
 145 150 155 160

Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val Ser  
 165 170 175

Gln Asn Tyr

<210> 45

<211> 186

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 45

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Gly Ala  
 20 25 30

Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp Glu Lys Ile  
 35 40 45

Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val  
 50 55 60

Trp Ala Ser Arg Glu Leu Glu Arg Gly Gln Leu Gln Pro Ser Leu Gln  
 65 70 75 80

Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu  
 85 90 95

Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp Thr Lys Glu Ala Leu  
 100 105 110

Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln



## Genphar.740.ST25.txt

<400> 47  
 atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg 60  
 ctccctggga tgttgatgat ctgtagtgtc cctatagtgc agaacatcca ggggcaaatg 120  
 gtacatcagg ccatatcacc tagaacttta aatgcatggg taaaagtagt agaagagaag 180  
 gctttcagcc cagaagtaat acccatgttt tcagcattat cagaaggagc caccaccaca 240  
 gatttaaaca ccatgctaaa cacagtgggg ggacatcaag cagccatgca aatgttaaaa 300  
 gagaccatca atgaggaagc tgcagaatgg gatagagtac atccagtgca tgcagggcct 360  
 attgcaccag gccagatgag agaaccaagg ggaagtgaca tagcaggaac tactagtacc 420  
 cttcaggaac aaataggatg gatgacaaat aatccaccta tcccagtagg agaaatttat 480  
 aaaagatgga taatcctggg attaaataaa atagtaagaa tgtatagccc taccagcatt 540  
 ctggacataa gacaaggacc aaaagaacct tttagagact atgtagaccg gttctataaa 600  
 actctaagag ccgagcaagc ttcacaggag gtaaaaaatt ggatgacaga aacctgtgtg 660  
 gtccaaaatg cgaaccacga ttgtaagact attttaaaag cattgggacc agcggctaca 720  
 ctagaagaaa tgatgacagc atgtcaggga gtaggaggac ccggccataa ggcaagagtt 780  
 ttgtaa 786

<210> 48

<211> 915

<212> DNA

<213> Human immunodeficiency virus type 1

<400> 48  
 atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg 60  
 ctccctggga tgttgatgat ctgtagtgtc cctatagtgc agaacatcca ggggcaaatg 120  
 gtacatcagg ccatatcacc tagaacttta aatgcatggg taaaagtagt agaagagaag 180  
 gctttcagcc cagaagtaat acccatgttt tcagcattat cagaaggagc caccaccaca 240  
 gatttaaaca ccatgctaaa cacagtgggg ggacatcaag cagccatgca aatgttaaaa 300  
 gagaccatca atgaggaagc tgcagaatgg gatagagtac atccagtgca tgcagggcct 360  
 attgcaccag gccagatgag agaaccaagg ggaagtgaca tagcaggaac tactagtacc 420  
 cttcaggaac aaataggatg gatgacaaat aatccaccta tcccagtagg agaaatttat 480  
 aaaagatgga taatcctggg attaaataaa atagtaagaa tgtatagccc taccagcatt 540  
 ctggacataa gacaaggacc aaaagaacct tttagagact atgtagaccg gttctataaa 600

Genphar.740.ST25.txt

|  |     |
|--|-----|
| actctaagag ccgagcaagc ttcacaggag gtaaaaaatt ggatgacaga aaccttggtg  | 660 |
| gtccaaaatg cgaaccaga ttgtaagact attttaaaag cattgggacc agcggctaca   | 720 |
| ctagaagaaa tgatgacagc atgtcagga gtaggaggac ccggccataa ggcaagagtt   | 780 |
| ttgttattca taatgatagt aggaggcttg gtaggtttaa gaatagtttt tgctgtactt  | 840 |
| tctgtagtga atagagtttag gcagggatat tcaccattat cgtttcagac ccacctccca | 900 |
| atccccgaggg gataa  | 915 |

&lt;210&gt; 49

&lt;211&gt; 232

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 49

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Pro | Ile | Val | Gln | Asn | Ile | Gln | Gly | Gln | Met | Val | His | Gln | Ala | Ile |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Arg | Thr | Leu | Asn | Ala | Trp | Val | Lys | Val | Val | Glu | Glu | Lys | Ala |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Ser | Pro | Glu | Val | Ile | Pro | Met | Phe | Ser | Ala | Leu | Ser | Glu | Gly | Ala |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Gln | Asp | Leu | Asn | Thr | Met | Leu | Asn | Thr | Val | Gly | Gly | His | Gln |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ala | Met | Gln | Met | Leu | Lys | Glu | Thr | Ile | Asn | Glu | Glu | Ala | Ala | Glu |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Asp | Arg | Val | His | Pro | Val | His | Ala | Gly | Pro | Ile | Ala | Pro | Gly | Gln |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Arg | Glu | Pro | Arg | Gly | Ser | Asp | Ile | Ala | Gly | Thr | Thr | Ser | Thr | Leu |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Glu | Gln | Ile | Gly | Trp | Met | Thr | Asn | Asn | Pro | Pro | Ile | Pro | Val | Gly |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ile | Tyr | Lys | Arg | Trp | Ile | Ile | Leu | Gly | Leu | Asn | Lys | Ile | Val | Arg |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Tyr | Ser | Pro | Thr | Ser | Ile | Leu | Asp | Ile | Arg | Gln | Gly | Pro | Lys | Glu |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

## Genphar.740.ST25.txt

Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu  
 165 170 175

Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val  
 180 185 190

Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro  
 195 200 205

Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly  
 210 215 220

Pro Gly His Lys Ala Arg Val Leu  
 225 230

<210> 50

<211> 261

<212> PRT

<213> Human immunodeficiency virus type 1

<400> 50

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Pro Ile  
 20 25 30

Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg  
 35 40 45

Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro  
 50 55 60

Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln  
 65 70 75 80

Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
 85 90 95

Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg  
 100 105 110

Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu

```

<400> 51
Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg
1      5      10      15
Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Pro Ile
20      25      30
Val Gln Asn Ile Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg
35      40      45

```

Genphar.740.ST25.txt

Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro  
50 55 60

Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln  
65 70 75 80

Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
85 90 95

Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Arg Glu Pro Arg  
100 105 110

Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly  
115 120 125

Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg  
130 135 140

Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr  
145 150 155 160

Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr  
165 170 175

Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu  
180 185 190

Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro  
195 200 205

Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu  
210 215 220

Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
225 230 235 240

Arg Val Leu Leu Phe Ile Met Ile Val Gly Gly Leu Val Gly Leu Arg  
245 250 255

Ile Val Phe Ala Val Leu Ser Val Val Asn Arg Val Arg Gln Gly Tyr  
260 265 270

Ser Pro Leu Ser Phe Gln Thr His Leu Pro Ile Pro Arg Gly  
275 280 285

<210> 52

<211> 3839



## Genphar.740.ST25.txt

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env/Tat

&lt;400&gt; 52

```

gaattctgca acaactgctg tttatccatt ttcagaattg ggtgtcgaca tagcagaata      60
ggcgttactc gacagaggag agcaagaaat ggagccagta gatcctagac tagagccctg      120
gaagcatcca ggaagtcagc ctaaaactgc ttgtaccaat tgctattgta aaaagtgttg      180
ctttcattgc caagtttggt tcataacaaa agccttaggc atctcctatg gcaggaagaa      240
gcggagacag cgacgaagac ctctcaagg cagtcagact catcaagttt ctctatcaaa      300
gcagtaagta gtacatgtaa tgcaacctat acaaatagca atagtagcat tagtagtagc      360
aataataata gcaatagttg tgtggtccat agtaatcata gaatatagga aaatattaag      420
acaaagaaaa atagacaggt taattgatag actaatagaa agagcagaag acagtggcaa      480
tgagagtgaa ggagaaatat cagcacttgt ggagatgggg gtggagatgg ggcaccatgc      540
tccttgggat gttgatgata tgtagtgcta cagaaaaatt gtgggtcaca gtctattatg      600
gggtacctgt gtggaaggaa gcaaccacca ctctattttg tgcatcagat gctaaagcat      660
atgatacaga ggtacataat gtttgggcca cacatgcctg tgtaccaca gacccaacc      720
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aattaacccc actctgtggt ggagctggta gttgtaacac ctcagtcatt acacaggcct      900
gtccaaaggt atcctttgag ccaattccca tacattattg tgccccggct ggttttgcga      960
ttctaaaatg taataataag acgttcaatg gaacaggacc atgtacaaat gtcagcacag     1020
tacaatgtac acatggaatt aggccagtag tatcaactca actgctgtta aatggcagtc     1080
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aaagtatccg tatccagaga ggaccaggga gagcatttgt tacaatagga aaaataggaa     1260
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tacgtatagg accaggacaa acattctatg caacagggtga tataataggg gatataagac     1380
aagcacattg ttgtacgaga cccaacaata atacaagaaa aagtataagg ataggaccag     1440
gacaagcatt ctatgcaaca ggagaaataa taggagatat aagacaagca cattgttgca     1500
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## Genphar.740.ST25.txt

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| caagaacaag tatacgata ggaccaggac aagtattcta tagaacagga gacataacag  | 1680 |
| gagatataag aaaagcatat tgtggatcct gtacaagacc caacaacaat acaagaaaaa | 1740 |
| gaatatcttt aggaccagga cgagtatttt atacagcagg agaaataata ggagacatca | 1800 |
| gaaaggcaca ttgttgtacc agacctata acaatacaag aaaaagtata acttttgcac  | 1860 |
| caggacaagc gctctatgca acaggtgaaa taataggaga tataagacaa gcacattgtc | 1920 |
| tcgggtgtac cagacctaac aacaatacaa gaaaaagtgt acgtatagga ccaggacaaa | 1980 |
| cattctatgc aacaggtgat ataatagggg atataagaca agcacattgt tgtacgagac | 2040 |
| ccaacaataa tacaagaaaa agtataagga taggaccagg acaagcattc tatgcaacag | 2100 |
| gagaaataat aggagatata agacaagcac attgttgcac aaggccctac aacaatataa | 2160 |
| gacaaaggac ccccatagga ctagggcaag cactctatac aacaagaaga atagaagata | 2220 |
| taagaagagc acattgttgt accagaccct ccaccaatac aagaacaagt atacgtatag | 2280 |
| gaccaggaca agtattctat agaacaggag acataacagg agatataaga aaagcatatt | 2340 |
| gtggatcctg tacaagaccc aacaacaata caagaaaaag aatatcttta ggaccaggac | 2400 |
| gagtatttta tacagcagga gaaataatag gagacatcag aaaggcacat tgttgtacca | 2460 |
| gacctataa caatacaaga aaaagtataa cttttgcacc aggacaagcg ctctatgcaa  | 2520 |
| caggtgaaat aataggagat ataagacaag cacattgtct cgggaacatt agtagagcaa | 2580 |
| aatggaataa cactttaaaa cagatagata gcaaattaag agaacaattt ggaaataata | 2640 |
| aaacaataat ctttaagcag tcctcaggag gggaccaga aattgtaacg cacagtttta  | 2700 |
| attgtggagg ggaatttttc tactgtatt caacacaact gttaataagt acttggttta  | 2760 |
| atagtacttg gagtactaaa gggcacaata aactgaagg aagtgcaca atcaccctcc   | 2820 |
| catgcagaat aaaacaaatt ataaacatgt ggcaggaagt aggaaaagca atgtatgcc  | 2880 |
| ctcccatcag tggacaaatt agatgttcat caaatattac agggctgcta ttaacaagag | 2940 |
| atggtggtaa tagcaacaat gagtccgaga tcttcagacc tggaggagga gatatgagg  | 3000 |
| acaattggag aagtgaatta tataaatata aagttagtaa aattgaacca ttaggagtag | 3060 |
| caccaccaa ggcaaagaga agagtgtgc agactagtgc agtgggaata ggagctttgt   | 3120 |
| tccttgggtt cttgggagca gcaggagca ctatgggcgc agcgtcaatg acgctgacgg  | 3180 |
| tacaggccag acaattattg tctggtatag tgcagcagca gaacaatttg ctgagggcta | 3240 |
| ttgaggcgca acagcatctg ttgcaactca cagtctgggg catcaagcag ctccaggcaa | 3300 |
| gaatcctggc tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggtgtct | 3360 |
| ctggaaaact catttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc | 3420 |

Genphar.740.ST25.txt

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|---|------|
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| caagcttaat acactcctta attgaagaat cgcaaaacca gcaagaaaag aatgaacaag | 3540 |
| aattattgga attagataaa tgggcaagtt tgtggaattg gtttaacata acaaattggc | 3600 |
| tgtgttatat aaaattattc ataatgatag taggaggctt ggtaggttta agaatagttt | 3660 |
| ttgctgtact ttctgtagt aatagagtta ggcagggata ttcaccatta tcgtttcaga  | 3720 |
| cccacctccc aatcccagg ggacccgaca ggcccgaagg aatagaagaa gaagggtggag | 3780 |
| agagagacag agacagatcc attcgattag tgaacggatc cttagcactt atctggtaa  | 3839 |

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<211> 1101

<212> PRT

<213> Artificial sequence

<220>

<223> Modified Env/Tat

<400> 53

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Arg | Val | Lys | Glu | Lys | Tyr | Gln | His | Leu | Trp | Arg | Trp | Gly | Trp | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Gly | Thr | Met | Leu | Leu | Gly | Met | Leu | Met | Ile | Cys | Ser | Ala | Thr | Glu |
|     |     |     | 20  |     |     |     | 25  |     |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Leu | Trp | Val | Thr | Val | Tyr | Tyr | Gly | Val | Pro | Val | Trp | Lys | Glu | Ala |
|     |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Thr | Thr | Leu | Phe | Cys | Ala | Ser | Asp | Ala | Lys | Ala | Tyr | Asp | Thr | Glu |
|     | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | His | Asn | Val | Trp | Ala | Thr | His | Ala | Cys | Val | Pro | Thr | Asp | Pro | Asn |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Glu | Val | Val | Leu | Val | Asn | Val | Thr | Glu | Asn | Phe | Asn | Met | Trp |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Asn | Asp | Met | Val | Glu | Gln | Met | His | Glu | Asp | Ile | Ile | Ser | Leu | Trp |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Gln | Ser | Leu | Lys | Pro | Cys | Val | Lys | Leu | Thr | Pro | Leu | Cys | Val | Gly |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

Genphar.740.ST25.txt  
 Ala Gly Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val  
 130 135 140  
 Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala  
 145 150 155 160  
 Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr  
 165 170 175  
 Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser  
 180 185 190  
 Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val Ile  
 195 200 205  
 Arg Ser Ala Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu  
 210 215 220  
 Asn Gln Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg  
 225 230 235 240  
 Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile  
 245 250 255  
 Gly Lys Ile Gly Asn Met Arg Gln Ala His Cys Leu Gly Cys Thr Arg  
 260 265 270  
 Pro Asn Asn Asn Thr Arg Lys Ser Val Arg Ile Gly Pro Gly Gln Thr  
 275 280 285  
 Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
 290 295 300  
 Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro  
 305 310 315 320  
 Gly Gln Ala Phe Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln  
 325 330 335  
 Ala His Cys Cys Thr Arg Pro Tyr Asn Asn Ile Arg Gln Arg Thr Pro  
 340 345 350  
 Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr Arg Arg Ile Glu Asp Ile  
 355 360 365  
 Arg Arg Ala His Cys Cys Thr Arg Pro Ser Thr Asn Thr Arg Thr Ser  
 370 375 380

## Genphar.740.ST25.txt

Ile Arg Ile Gly Pro Gly Gln Val Phe Tyr Arg Thr Gly Asp Ile Thr  
 385 390 395 400  
 Gly Asp Ile Arg Lys Ala Tyr Cys Gly Ser Cys Thr Arg Pro Asn Asn  
 405 410 415  
 Asn Thr Arg Lys Arg Ile Ser Leu Gly Pro Gly Arg Val Phe Tyr Thr  
 420 425 430  
 Ala Gly Glu Ile Ile Gly Asp Ile Arg Lys Ala His Cys Cys Thr Arg  
 435 440 445  
 Pro Asn Asn Asn Thr Arg Lys Ser Ile Thr Phe Ala Pro Gly Gln Ala  
 450 455 460  
 Leu Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
 465 470 475 480  
 Leu Gly Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Val Arg Ile  
 485 490 495  
 Gly Pro Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile  
 500 505 510  
 Arg Gln Ala His Cys Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser  
 515 520 525  
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 Gly Asp Ile Arg Gln Ala His Cys Cys Thr Arg Pro Tyr Asn Asn Ile  
 545 550 555 560  
 Arg Gln Arg Thr Pro Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr Arg  
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 Arg Ile Glu Asp Ile Arg Arg Ala His Cys Cys Thr Arg Pro Ser Thr  
 580 585 590  
 Asn Thr Arg Thr Ser Ile Arg Ile Gly Pro Gly Gln Val Phe Tyr Arg  
 595 600 605  
 Thr Gly Asp Ile Thr Gly Asp Ile Arg Lys Ala Tyr Cys Gly Ser Cys  
 610 615 620  
 Thr Arg Pro Asn Asn Asn Thr Arg Lys Arg Ile Ser Leu Gly Pro Gly  
 625 630 635 640

## Genphar.740.ST25.txt

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 His Cys Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Thr Phe  
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 Ala Pro Gly Gln Ala Leu Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile  
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 Arg Gln Ala His Cys Leu Gly Asn Ile Ser Arg Ala Lys Trp Asn Asn  
 690 695 700  
 Thr Leu Lys Gln Ile Asp Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn  
 705 710 715 720  
 Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val  
 725 730 735  
 Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr  
 740 745 750  
 Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Lys Gly  
 755 760 765  
 Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile  
 770 775 780  
 Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala  
 785 790 795 800  
 Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu  
 805 810 815  
 Leu Leu Thr Arg Asp Gly Gly Asn Ser Asn Asn Glu Ser Glu Ile Phe  
 820 825 830  
 Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr  
 835 840 845  
 Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys  
 850 855 860  
 Ala Lys Arg Arg Val Val Gln Thr Ser Ala Val Gly Ile Gly Ala Leu  
 865 870 875 880  
 Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser

Genphar.740.ST25.txt

885                      890                      895

Met Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln  
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Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu  
                     915                      920                      925

Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala  
                     930                      935                      940

Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys  
                     945                      950                      955                      960

Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp  
                     965                      970                      975

Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu  
                     980                      985                      990

Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile  
                     995                      1000                      1005

Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu  
                     1010                      1015                      1020

Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr  
                     1025                      1030                      1035

Asn Trp Leu Trp Tyr Ile Lys Leu Phe Ile Met Ile Val Gly Gly  
                     1040                      1045                      1050

Leu Val Gly Leu Arg Ile Val Phe Ala Thr His Leu Pro Ile Pro  
                     1055                      1060                      1065

Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu  
                     1070                      1075                      1080

Arg Asp Arg Asp Arg Ser Ile Arg Leu Val Asn Gly Ser Leu Ala  
                     1085                      1090                      1095

Leu Ile Trp  
                     1100

&lt;210&gt; 54

&lt;211&gt; 4040

Genphar.740.ST25.txt

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env/Tat/Rev

&lt;400&gt; 54

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| gaagcatcca ggaagtcagc ctaaaactgc ttgtaccaat tgctattgta aaaagtgttg  | 180  |
| ctttcattgc caagtttggt tcataacaaa agccttaggc atctcctatg gcaggaagaa  | 240  |
| gcggagacag cgacgaagac ctctcaagg cagtcagact catcaagttt ctctatcaaa   | 300  |
| gcagtaagta gtacatgtaa tgcaacctat acaaatagca atagtagcat tagtagtagc  | 360  |
| aataataata gcaatagttg tgtgggccat agtaatcata gaatatagga aaatattaag  | 420  |
| acaaagaaaa atagacaggt taattgatag actaatagaa agagcagaag acagtggcaa  | 480  |
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| tagaacagat gcatgaggat ataatcagtt tatgggatca aagcctaaag ccatgtgtaa  | 840  |
| aattaacccc actctgtgtt ggagctggta gttgtaacac ctcagtcatt acacaggcct  | 900  |
| gtccaaagggt atcctttgag ccaattccca tacattattg tgccccggct ggttttgcga | 960  |
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| tggcagaaga agaggtagta attagatctg ccaatttcac agacaatgct aaaaccataa  | 1140 |
| tagtacagct gaaccaatct gtagaaatta attgtacaag acccaacaac aatacaagaa  | 1200 |
| aaagtatccg tatccagaga ggaccaggga gagcatttgt tacaatagga aaaataggaa  | 1260 |
| atatgagaca agcacattgt ctcggtgta ccagacctaa caacaatata agaaaaagtg   | 1320 |
| tacgtatagg accaggacaa acattctatg caacagggtga tataataggg gatataagac | 1380 |
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| gacaagcatt ctatgcaaca ggagaaataa taggagatat aagacaagca cattgttgca  | 1500 |
| caaggcccta caacaatata agacaaagga ccccatagg actagggcaa gcactctata   | 1560 |



Genphar.740.ST25.txt

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|--|------|
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| gagatataag aaaagcatat tgtggatcct gtacaagacc caacaacaat acaagaaaaa  | 1740 |
| gaatatcttt aggaccagga cgagtatttt atacagcagg agaaataata ggagacatca  | 1800 |
| gaaaggcaca ttgttgtacc agacctaata acaatacaag aaaaagtata acttttgcac  | 1860 |
| caggacaagc gctctatgca acaggtgaaa taataggaga tataagacaa gcacattgtc  | 1920 |
| tcgggtgtac cagacctaac aacaatacaa gaaaaagtgt acgtatagga ccaggacaaa  | 1980 |
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| ccaacaataa tacaagaaaa agtataagga taggaccagg acaagcattc tatgcaacag  | 2100 |
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| aaacaataat ctttaagcag tcctcaggag gggaccacga aattgtaacg cacagtttta  | 2700 |
| attgtggagg ggaatttttc tactgttaatt caacacaact gttaataagt acttggttta | 2760 |
| atagtacttg gagtactaaa ggggtcaaata aactgaagg aagtgcaca atcacctcc    | 2820 |
| catgcagaat aaaacaaatt ataaacatgt ggcaggaagt aggaaaagca atgtatgccc  | 2880 |
| ctcccatcag tggacaaatt agatgttcat caaatattac agggctgcta ttaacaagag  | 2940 |
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| acaattggag aagtgaatta tataaatata aagtagtaaa aattgaacca ttaggagtag  | 3060 |
| caccaccaa ggcaaagaga agagtgggtc agactagtgc agtgggaata ggagctttgt   | 3120 |
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| tacaggccag acaattattg tctgatatag tgcagcagca gaacaatttg ctgagggcta  | 3240 |
| ttgaggcgca acagcatctg ttgcaactca cagtctggg catcaaacag ctccaggcaa   | 3300 |
| gaatcctggc tgtggaaaga tacctaaagg atcaacagct cctggggatt tggggttgct  | 3360 |
| ctggaaaact cttttgcacc actgctgtgc cttggaatgc tagttggagt aataaatctc  | 3420 |
| tggaacagat ttggaataac atgacctgga tggagtggga cagagaaatt aacaattaca  | 3480 |

## Genphar.740.ST25.txt

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cgaggattgt ggaacttctg ggacgcaggg ggtgggaagc cctcaaatat tgggtggaatc 3960
tcctacagta ttggagtcag gaactaaaga atagtgctgt taacttgctc aatgccacag 4020
ccatagcagt agctgagtaa 4040

```

&lt;210&gt; 55

&lt;211&gt; 1186

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Modified Env/Tat/Rev

&lt;400&gt; 55

Met Arg Val Lys Glu Lys Tyr Gln His Leu Trp Arg Trp Gly Trp Arg  
 1 5 10 15

Trp Gly Thr Met Leu Leu Gly Met Leu Met Ile Cys Ser Ala Thr Glu  
 20 25 30

Lys Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala  
 35 40 45

Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu  
 50 55 60

Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn  
 65 70 75 80

Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn Phe Asn Met Trp  
 85 90 95

Lys Asn Asp Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp

Genphar.740.ST25.txt

100 105 110

Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Gly  
 115 120 125

Ala Gly Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val  
 130 135 140

Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe Ala  
 145 150 155 160

Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr  
 165 170 175

Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser  
 180 185 190

Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val Ile  
 195 200 205

Arg Ser Ala Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu  
 210 215 220

Asn Gln Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr Arg  
 225 230 235 240

Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile  
 245 250 255

Gly Lys Ile Gly Asn Met Arg Gln Ala His Cys Leu Gly Cys Thr Arg  
 260 265 270

Pro Asn Asn Asn Thr Arg Lys Ser Val Arg Ile Gly Pro Gly Gln Thr  
 275 280 285

Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
 290 295 300

Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gly Pro  
 305 310 315 320

Gly Gln Ala Phe Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln  
 325 330 335

Ala His Cys Cys Thr Arg Pro Tyr Asn Asn Ile Arg Gln Arg Thr Pro  
 340 345 350

Genphar.740.ST25.txt

Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr Arg Arg Ile Glu Asp Ile  
 355 360 365

Arg Arg Ala His Cys Cys Thr Arg Pro Ser Thr Asn Thr Arg Thr Ser  
 370 375 380

Ile Arg Ile Gly Pro Gly Gln Val Phe Tyr Arg Thr Gly Asp Ile Thr  
 385 390 395 400

Gly Asp Ile Arg Lys Ala Tyr Cys Gly Ser Cys Thr Arg Pro Asn Asn  
 405 410 415

Asn Thr Arg Lys Arg Ile Ser Leu Gly Pro Gly Arg Val Phe Tyr Thr  
 420 425 430

Ala Gly Glu Ile Ile Gly Asp Ile Arg Lys Ala His Cys Cys Thr Arg  
 435 440 445

Pro Asn Asn Asn Thr Arg Lys Ser Ile Thr Phe Ala Pro Gly Gln Ala  
 450 455 460

Leu Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile Arg Gln Ala His Cys  
 465 470 475 480

Leu Gly Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Val Arg Ile  
 485 490 495

Gly Pro Gly Gln Thr Phe Tyr Ala Thr Gly Asp Ile Ile Gly Asp Ile  
 500 505 510

Arg Gln Ala His Cys Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser  
 515 520 525

Ile Arg Ile Gly Pro Gly Gln Ala Phe Tyr Ala Thr Gly Glu Ile Ile  
 530 535 540

Gly Asp Ile Arg Gln Ala His Cys Cys Thr Arg Pro Tyr Asn Asn Ile  
 545 550 555 560

Arg Gln Arg Thr Pro Ile Gly Leu Gly Gln Ala Leu Tyr Thr Thr Arg  
 565 570 575

Arg Ile Glu Asp Ile Arg Arg Ala His Cys Cys Thr Arg Pro Ser Thr  
 580 585 590

Asn Thr Arg Thr Ser Ile Arg Ile Gly Pro Gly Gln Val Phe Tyr Arg  
 595 600 605

## Genphar.740.ST25.txt

Thr Gly Asp Ile Thr Gly Asp Ile Arg Lys Ala Tyr Cys Gly Ser Cys  
 610 615 620  
 Thr Arg Pro Asn Asn Asn Thr Arg Lys Arg Ile Ser Leu Gly Pro Gly  
 625 630 635 640  
 Arg Val Phe Tyr Thr Ala Gly Glu Ile Ile Gly Asp Ile Arg Lys Ala  
 645 650 655  
 His Cys Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Thr Phe  
 660 665 670  
 Ala Pro Gly Gln Ala Leu Tyr Ala Thr Gly Glu Ile Ile Gly Asp Ile  
 675 680 685  
 Arg Gln Ala His Cys Leu Gly Asn Ile Ser Arg Ala Lys Trp Asn Asn  
 690 695 700  
 Thr Leu Lys Gln Ile Asp Ser Lys Leu Arg Glu Gln Phe Gly Asn Asn  
 705 710 715 720  
 Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly Asp Pro Glu Ile Val  
 725 730 735  
 Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys Asn Ser Thr  
 740 745 750  
 Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Lys Gly  
 755 760 765  
 Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile  
 770 775 780  
 Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala  
 785 790 795 800  
 Pro Pro Ile Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu  
 805 810 815  
 Leu Leu Thr Arg Asp Gly Gly Asn Ser Asn Asn Glu Ser Glu Ile Phe  
 820 825 830  
 Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr  
 835 840 845  
 Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Val Ala Pro Thr Lys  
 850 855 860

## Genphar.740.ST25.txt

Ala Lys Arg Arg Val Val Gln Thr Ser Ala Val Gly Ile Gly Ala Leu  
 865 870 875 880  
 Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Cys Thr Ser  
 885 890 895  
 Met Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Asp Ile Val Gln  
 900 905 910  
 Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu  
 915 920 925  
 Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Ile Leu Ala  
 930 935 940  
 Val Glu Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys  
 945 950 955 960  
 Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala Ser Trp  
 965 970 975  
 Ser Asn Lys Ser Leu Glu Gln Ile Trp Asn Asn Met Thr Trp Met Glu  
 980 985 990  
 Trp Asp Arg Glu Ile Asn Asn Tyr Thr Ser Leu Ile His Ser Leu Ile  
 995 1000 1005  
 Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Gln Glu Leu Leu  
 1010 1015 1020  
 Glu Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr  
 1025 1030 1035  
 Asn Trp Leu Trp Tyr Ile Lys Leu Phe Ile Met Ile Val Gly Gly  
 1040 1045 1050  
 Leu Val Gly Leu Arg Ile Val Phe Ala Val Leu Ser Ile Val Asn  
 1055 1060 1065  
 Arg Val Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr His Leu  
 1070 1075 1080  
 Pro Ile Pro Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu  
 1085 1090 1095  
 Gly Gly Glu Arg Asp Arg Asp Arg Ser Ile Arg Leu Val Asn Gly

Genphar.740.ST25.txt

1100                      1105                      1110

Ser Leu Ala Leu Ile Trp Asp Asp Leu Arg Ser Leu Cys Leu Phe  
 1115                      1120                      1125

Ser Tyr His Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg Ile  
 1130                      1135                      1140

Val Glu Leu Leu Gly Arg Arg Gly Trp Glu Ala Leu Lys Tyr Trp  
 1145                      1150                      1155

Trp Asn Leu Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala  
 1160                      1165                      1170

Val Asn Leu Leu Asn Ala Thr Ala Ile Ala Val Ala Glu  
 1175                      1180                      1185

&lt;210&gt; 56

&lt;211&gt; 507

&lt;212&gt; DNA

&lt;213&gt; Human immunodeficiency virus type 1

<400> 56  
 atgttcttta gggaagatct ggccttccta caaggggaagg ccaggggaatt ttcttcagag 60  
 cagaccagag ccaacagccc caccatttct tcagagcaga ccagagccaa cagccccacc 120  
 agaagagagc ttcaggtctg gggtagagac aacaactccc cctcagaagc aggagccgat 180  
 agacaaggaa ctgtatcctt taacttcctt cagatcactc tttggcaacg acccctcgtc 240  
 acaataaaga taggggggca actaaaggaa gctctattag atacaggagc agatgatata 300  
 gtattagaag aaatgagttt gccaggaaga tggaaccaa aaatgatagg gggaattgga 360  
 ggttttatca aagtaagaca gtatgatcag atactcatag aaatctgtgg acataaagct 420  
 ataggtacag tattagtagg acctacacct gtcaacataa ttggaagaaa tctgttgact 480  
 cagattgggt gcactttaaa tttttaa 507

&lt;210&gt; 57

&lt;211&gt; 168

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus type 1

Genphar.740.ST25.txt

&lt;400&gt; 57

Met Phe Phe Arg Glu Asp Leu Ala Phe Leu Gln Gly Lys Ala Arg Glu  
 1 5 10 15

Phe Ser Ser Glu Gln Thr Arg Ala Asn Ser Pro Thr Ile Ser Ser Glu  
 20 25 30

Gln Thr Arg Ala Asn Ser Pro Thr Arg Arg Glu Leu Gln Val Trp Gly  
 35 40 45

Arg Asp Asn Asn Ser Pro Ser Glu Ala Gly Ala Asp Arg Gln Gly Thr  
 50 55 60

Val Ser Phe Asn Phe Pro Gln Ile Thr Leu Trp Gln Arg Pro Leu Val  
 65 70 75 80

Thr Ile Lys Ile Gly Gly Gln Leu Lys Glu Ala Leu Leu Asp Thr Gly  
 85 90 95

Ala Asp Asp Thr Val Leu Glu Glu Met Ser Leu Pro Gly Arg Trp Lys  
 100 105 110

Pro Lys Met Ile Gly Gly Ile Gly Gly Phe Ile Lys Val Arg Gln Tyr  
 115 120 125

Asp Gln Ile Leu Ile Glu Ile Cys Gly His Lys Ala Ile Gly Thr Val  
 130 135 140

Leu Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn Leu Leu Thr  
 145 150 155 160

Gln Ile Gly Cys Thr Leu Asn Phe  
 165

&lt;210&gt; 58

&lt;211&gt; 1800

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Gag-PI

&lt;400&gt; 58

atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60



Genphar.740.ST25.txt

|   |      |
|---|------|
| ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag | 120  |
| ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata | 180  |
| ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat | 240  |
| acagtagcaa cctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct  | 300  |
| ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct | 360  |
| gacacaggac acagcagtca ggtcagccaa aattacccta tagtgcagaa catccagggg | 420  |
| caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa | 480  |
| gagaaggctt tcagcccaga agtaataccc atgttttcag cattatcaga aggagccacc | 540  |
| ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatg | 600  |
| ttaaagaga ccatcaatga ggaagctgca gaatgggata gagtacatcc agtgcagtca  | 660  |
| gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact | 720  |
| agtacccttc aggaacaaat aggatggatg acaataatc cacctatccc agtaggagaa  | 780  |
| atttataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc | 840  |
| agcattcttg acataagaca aggacaaaa gaacctttta gagactatgt agaccggttc  | 900  |
| tataaaactc taagagccga gcaagcttca caggaggtaa aaaattggat gacagaaacc | 960  |
| ttgttggtcc aaaatgcgaa ccagattgt aagactattt taaaagcatt gggaccagcg  | 1020 |
| gctacactag aagaaatgat gacagcatgt caggagtag gaggaccgg ccataaggca   | 1080 |
| agagtttttg ctgaagcaat gagccaagta acaatacag ctaccataat gatgcagaga  | 1140 |
| ggcaatttta ggaaccaaag aaagatggtt aagtgtttca attgtggca agaagggcac  | 1200 |
| acagccagaa attgcagggc ccctaggaaa aagggtgtt ggaaatgtgg aaaggaagga  | 1260 |
| caccaaataa aagattgtac tgagagacag gctaatttct ttagggaaga tctggccttc | 1320 |
| ctacaagga aggccagga attttcttca gagcagacca gagccaacag cccaccatt    | 1380 |
| tcttcagagc agaccagagc caacagcccc accagaagag agcttcaggt ctggggtaga | 1440 |
| gacaacaact cccctcaga agcaggagcc gatagacaag gaactgtatc ctttaacttc  | 1500 |
| cctcagatca ctctttggca acgacccctc gtcacaataa agataggggg gcaactaaag | 1560 |
| gaagctctat tagatacagg agcagatgat acagtattag aagaaatgag tttgccagga | 1620 |
| agatggaaac caaaaatgat agggggaatt ggaggtttta tcaaagtaag acagtatgat | 1680 |
| cagatactca tagaaatctg tggacataaa gctataggta cagtattagt aggacctaca | 1740 |
| cctgtcaaca taattggaag aaatctgttg actcagattg gttgcacttt aaatttttaa | 1800 |

&lt;210&gt; 59

&lt;211&gt; 599

Genphar.740.ST25.txt

&lt;212&gt; PRT

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; Gag-PI

&lt;400&gt; 59

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Arg Trp  
 1 5 10 15

Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys  
 20 25 30

His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro  
 35 40 45

Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu  
 50 55 60

Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn  
 65 70 75 80

Thr Val Ala Thr Leu Tyr Cys Val His Gln Arg Ile Glu Ile Lys Asp  
 85 90 95

Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys  
 100 105 110

Lys Lys Ala Gln Gln Ala Ala Ala Asp Thr Gly His Ser Ser Gln Val  
 115 120 125

Ser Gln Asn Tyr Pro Ile Val Gln Asn Ile Gln Gly Gln Met Val His  
 130 135 140

Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu  
 145 150 155 160

Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser  
 165 170 175

Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly  
 180 185 190

Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu  
 195 200 205

## Genphar.740.ST25.txt

Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala  
 210 215 220  
 Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr  
 225 230 235 240  
 Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile  
 245 250 255  
 Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys  
 260 265 270  
 Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
 275 280 285  
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu  
 290 295 300  
 Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr  
 305 310 315 320  
 Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala  
 325 330 335  
 Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly  
 340 345 350  
 Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser  
 355 360 365  
 Gln Val Thr Asn Thr Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg  
 370 375 380  
 Asn Gln Arg Lys Met Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His  
 385 390 395 400  
 Thr Ala Arg Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys  
 405 410 415  
 Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn  
 420 425 430  
 Phe Phe Arg Glu Asp Leu Ala Phe Leu Gln Gly Lys Ala Arg Glu Phe  
 435 440 445  
 Ser Ser Glu Gln Thr Arg Ala Asn Ser Pro Thr Ile Ser Ser Glu Gln  
 450 455 460

## Genphar.740.ST25.txt

Thr Arg Ala Asn Ser Pro Thr Arg Arg Glu Leu Gln Val Trp Gly Arg  
465 470 475 480

Asp Asn Asn Ser Pro Ser Glu Ala Gly Ala Asp Arg Gln Gly Thr Val  
485 490 495

Ser Phe Asn Phe Pro Gln Ile Thr Leu Trp Gln Arg Pro Leu Val Thr  
500 505 510

Ile Lys Ile Gly Gly Gln Leu Lys Glu Ala Leu Leu Asp Thr Gly Ala  
515 520 525

Asp Asp Thr Val Leu Glu Glu Met Ser Leu Pro Gly Arg Trp Lys Pro  
530 535 540

Lys Met Ile Gly Gly Ile Gly Gly Phe Ile Lys Val Arg Gln Tyr Asp  
545 550 555 560

Gln Ile Leu Ile Glu Ile Cys Gly His Lys Ala Ile Gly Thr Val Leu  
565 570 575

Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn Leu Leu Thr Gln  
580 585 590

Ile Gly Cys Thr Leu Asn Phe  
595

<210> 60

<211> 51

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 60

aaatcaaccg gaattgaatt ccctcgggtg taccagacct aacaacaata c

51

<210> 61

<211> 42

<212> DNA

<213> Artificial sequence

Genphar.740.ST25.txt

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 61

attgttgggt ctgtacaac aatgtgcttg tcttatatcc cc

42

&lt;210&gt; 62

&lt;211&gt; 41

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 62

ggggatataa gacaagcaca ttgtacgaga cccaacaata c

41

&lt;210&gt; 63

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 63

gttgtagggc cttgtgcaac aatgtgcttg tcttatatc

39

&lt;210&gt; 64

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 64

gatataagac aagcacattg ttgcacaagg ccctacaac

39

## Genphar.740.ST25.txt

<210> 65

<211> 36

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 65

ggtaggagggt ctggtacaac aatgtgctct tcttat

36

<210> 66

<211> 36

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 66

ataagaagag cacattgttg taccagaccc tccacc

36

<210> 67

<211> 47

<212> DNA

<213> Artificial sequence

<220>

<223> PCR primer

<400> 67

gtattgttgt tgggtcttgt acaacaatat gcttttctta tatctcc

47

<210> 68

<211> 47

<212> DNA

<213> Artificial sequence

Genphar.740.ST25.txt

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 68

ggagatataa gaaaagcata ttgtgtgaca agacccaaca acaatac

47

&lt;210&gt; 69

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 69

gttattaggt ctggtacaac aatgtgcctt tctgatgtc

39

&lt;210&gt; 70

&lt;211&gt; 39

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 70

gacatcagaa aggcacattg ttgtaccaga cctaataac

39

&lt;210&gt; 71

&lt;211&gt; 54

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; PCR primer

&lt;400&gt; 71

aataaactag tctagacccc cgagtctaga acaatgtgct tgtcttatat ctcc

54

## Genphar.740.ST25.txt

&lt;210&gt; 72

&lt;211&gt; 7

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; MMLV SD site

&lt;400&gt; 72

aggttaag

7

&lt;210&gt; 73

&lt;211&gt; 9

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; MMLV SA site

&lt;400&gt; 73

ctgctgcag

9

&lt;210&gt; 74

&lt;211&gt; 90

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding gp120 signal peptide

&lt;400&gt; 74

atgagagtga aggagaaata tcagcacttg tggagatggg ggtggagatg gggcaccatg 60

ctccttggga tgttgatgat ctgtagtgct 90

&lt;210&gt; 75

&lt;211&gt; 129

&lt;212&gt; DNA



Genphar.740.ST25.txt

&lt;213&gt; Artificial sequence

&lt;220&gt;

&lt;223&gt; DNA encoding gp41 transmembrane domain

&lt;400&gt; 75

ttattcataa tgatagtagg aggcttggtg ggtttaagaa tagtttttgc tgtactttct 60

gtagtgaata gagttaggca gggatattca ccattatcgt ttcagacca cctcccaatc 120

ccgagggga 129